ELECTRONIC SUPPLEMENTARY INFORMATION

Toxicity, bioaccumulation and biotransformation of Cu oxide nanoparticles in *Daphnia magna*

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Acute and chronic dose-response assays

Table S1 presents the concentrations used for the acute toxicity test with *Daphnia magna*. Table S2 shows the concentrations used for the chronic assays and the measured parameters such as mortality, number of broods, average reproduction and total number of neonates.

Table S1. Concentrations and pH of $CuSO_4$ and nCuO solutions used for determining dose-response curves for the acute toxicity to *Daphnia magna*.

Treatment	**[ ] mg Cu L^{-1} / pH**											
	[ ]	**pH**	**[ ]**	**pH**	**[ ]**	**pH**	**[ ]**	**pH**	**[ ]**	**pH**	**[ ]**	**pH**
$CuSO_4$	0	7.4	0.04	7.4	0.08	7.4	0.16	7.4	0.24	7.3	0.32	7.3
nCuO 25 nm	0	7.3	0.03 1	7.3	0066	7.3	0.1	7.3	0.13	7.3	0.16	7.3
nCuO 40 nm	0	7.2	1.5	7.1	2.5	7.2	3.5	7.2	4.5	7.3	5	7.3
nCuO 80 nm	0	7.2	0.32	7.1	1.6	7.1	3.2	7	10	7	16	7

Calculation of average number of broods and reproduction

The average number of broods and reproduction were calculated as shown below:

Average of broods = total number of broods/number of fertile daphnids;

Average of reproduction = average of (sum of n° neonates generated from a fertile daphnid/n° of broods from a fertile daphnid)

Table S2. Mortality, number of broods, average and cumulative reproduction of *Daphnia magna* exposed for 21 days to different nCuO and $CuSO_4$ concentrations. The control treatment corresponds to the feed solution.

Treatment (mg Cu L^{-1})	**pH**	**% mortality**	**Average of broods**	**Day of 1st brood**	**Average reproduction**	**Total number of neonates**
Control	7.4	0	4.2	8th	25.0	1019
nCuO 25 nm, (0.01248)	7.3	50	4.4	10th	5.8	125
nCuO n 25 nm, (0.00624)		40	4	10th	7.29	175
nCuO 25 nm, (0.00312)		30	3.71	8th	9.61	272
nCuO 25 nm, (0.00156)	7.2	10	3.77	8th	11.9	408
nCuO 40 nm, (1.90)	7.2	40	3.33	9th	23.7	719
nCuO 40 nm, (1.17)		30	3.77	8th	21.2	831
nCuO 40 nm, (0.585)		20	5.4	8th	15.2	837
nCuO 40 nm, (0.292)	7.2	10	4.3	8th	19.9	877
nCuO 80 nm, (1.86)	7.2	30	4.44	8th	15.7	678
nCuO 80 nm, (1.13)		30	4.44	8th	20.1	790
nCuO 80 nm, (0.565)		30	4.70	8th	20.1	918
nCuO 80 nm, (0.282)	7.3	10	4.4	8th	20.8	955
$CuSO_4$ (0.00128)	7.4	80	4.50	10th	9.42	77
$CuSO_4$ (0.00064)		40	4.33	9th	6.02	157
$CuSO_4$ (0.00032)		30	3.66	8th	9.19	206
$CuSO_4$ (0.00016)	7.4	10	5.11	8th	10.6	474

X-ray fluorescence microanalysis (µ-XRF) and X-ray absorption near the edge structure microanalysis (µ-XANES)

Fig. S1 (A) presents the setup used for the µ-XRF measurements in the benchtop equipment and (B) shows the sample holder for the µ-XANES. For µ-XANES, it was necessary to cover daphnids with Ultralene™ to avoid dehydration. This film is made of carbon and its composition and thickness do not interfere with the analysis.

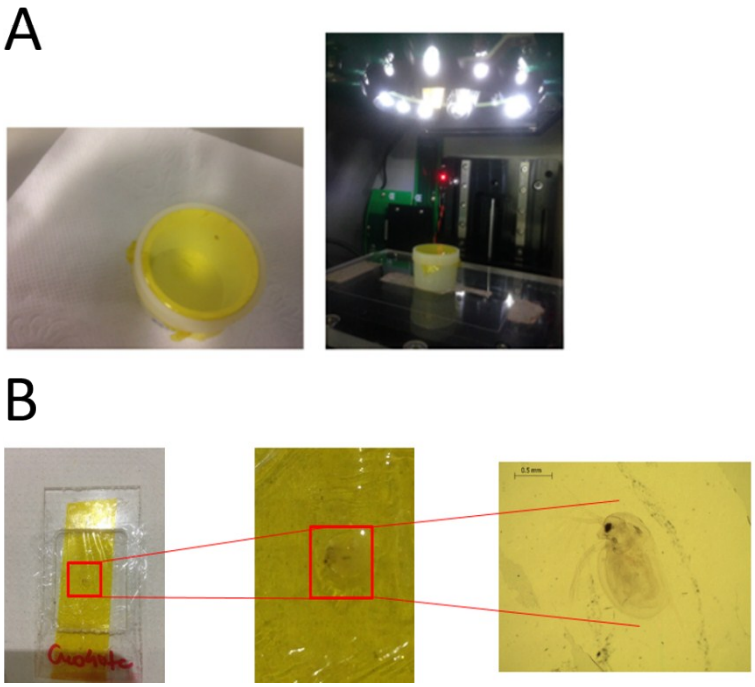

Fig. S1. (A) *Daphnia magna* on top of a Kapton™ (polyamide) film prepared for µ-XRF analysis, (B) Scheme of a sample of *D. magna* covered by a 4 µm Ultralene™ (polyethylene) film for XANES.

Nanoparticle and dispersion characterization

Fig. S2 shows the crystal structure of the differently sized nCuO. The graph was determined by Cu-Kα radiation X-ray diffraction (XRD) using a PM 1877 diffractometer (Philips, Netherlands).

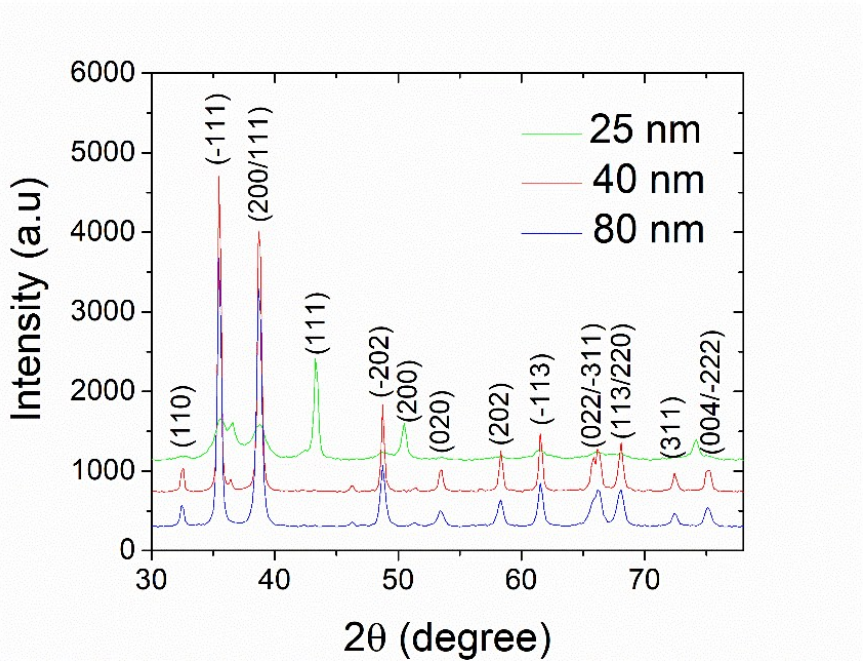

Fig. S2. XRD patterns for 25, 40 and 80 nm nCuO.

Micrograph images for nCuO dispersions were prepared to determine particle size and shape (Fig. S3). nCuO aqueous dispersions were prepared in deionized water and isopropanol (1:1) at 10 mg Cu L^{-1}. Micrograph images were recorded using a JEM-1011 transmission electronic microscope (Carl Zeiss AG, Germany) operating at 60 KV with the scales of electromicrographs printed directly.

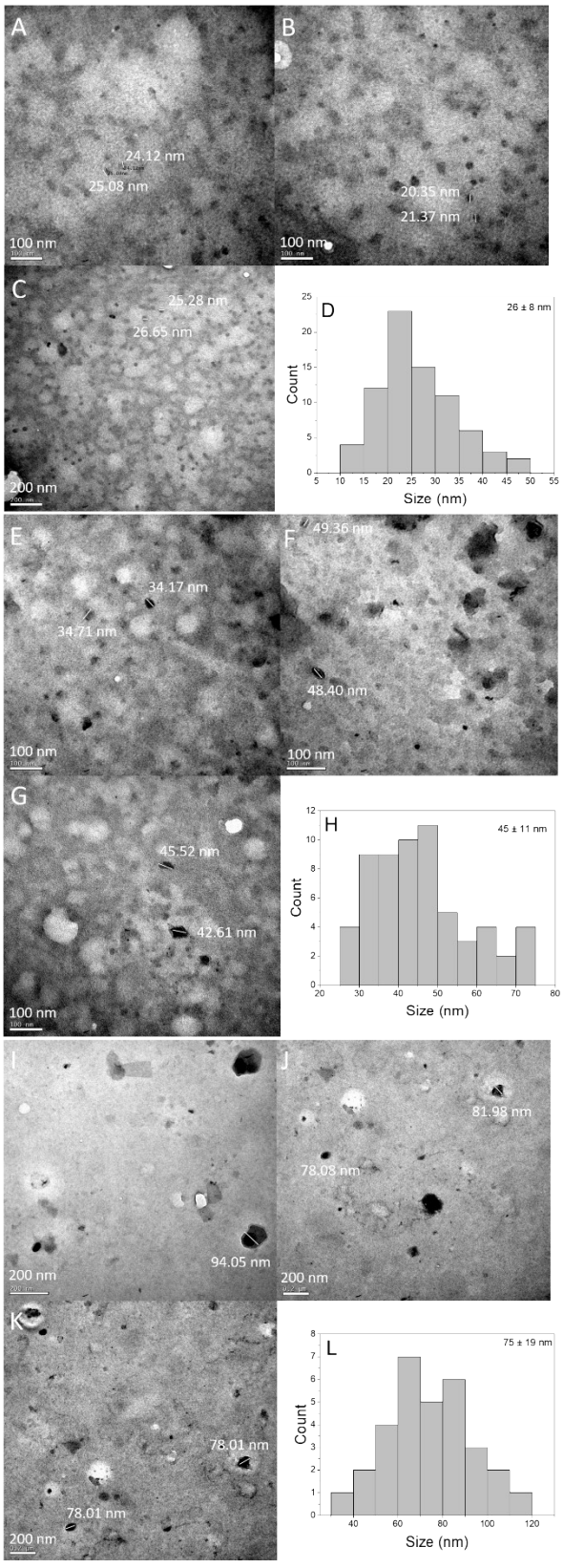

Fig. S3. Characterization of dispersions of nCuO at 10 mg Cu L^{-1} (50% deionized water and 50 % isopropanol) of (A, B, C) 25 nm, (E, F, G) 40 nm and (I, J, K) 80 nm by transmission electronic microscopy (TEM). (D, H, L) histograms showing the size distribution of nanoparticle counts for nCuO 25 nm, 40 nm and 80 nm, respectively.

Dissolution

We dripped 15 µL of supernatant (see the Nanoparticle and dispersion characterization section of the main manuscript) of nCuO dispersions and $CuSO_4$ solutions at 100 and 1000 Cu L^{-1} in a 6.3 window cuvette assembled with a five micrometer thick polypropylene film and dried the samples at 60°C in a laboratory oven. This procedure was repeated twice and samples were measured in triplicate using a rhodium X-ray tube operating at 50 kV. Spectra were acquired by a Si (Li) detector during 200 s. The quantification was made using external standard calibration and Ga as the internal standard, and using the formula shown below the LOQ was calculated. For the soluble concentration of Cu in deionized water and culture medium, the LOQ was 0.18 and 0.11 mg L^{-1}, respectively. Measurements were performed in triplicate in deionized water and culture medium[1].

$$LOQ = \frac{10\sqrt{BG}(cps)}{time\ (s)\ x\ electric\ current\ (\mu A)}$$

For zeta potential and dynamic light scattering (DLS) analysis, dispersions of nCuO at 100 mg Cu L^{-1} were prepared as describe for TEM analysis. The measurements were carried out using a Zetasizer Nano (Malvern Instruments, U.K) and the data are presented in Table 1 of the main manuscript.

Cu chemical speciation in solution based on Geochem simulation

Using the Geochem software it was possible to simulate the reactions between main solutions of the culture medium of *D. magna*, according ABNT 12713 (2016), with $CuSO_4$. The software works with the properties of the daphnids culture medium, which had a pH of 7-7.5.

In addition to $CuSO_4$, the following salt concentrations were used as input for the test solutions: KCl: 5.8 x 10^{-3} g L^{-1}; $MgSO_4.7H_2O$: 0.1233 g L^{-1}; $CaCl_2.2H_2O$: 0.2940 g L^{-1}; K_2HPO_4: 1.84 x 10^{-4} g L^{-1}; H_3BO_3: 2.85 x 10^{-3} g L^{-1}; Na_2MoO_4: 6.3 x 10^{-5} g L^{-1}; $MnCl_2.4H_2O$: 7.21 x 10^{-4} g L^{-1}; $SrCl_2.6H_2O$: 3.04 x 10^{-4} g L^{-1}; LiCl: 6.12 x 10^{-4} g L^{-1}; RbCl: 1.4 x 10^{-4} g L^{-1}; $CuCl_2.H_2O$: 3.35 x 10^{-5} g L^{-1}; $ZnCl_2$: 2.6 x 10^{-5} g L^{-1}; $Fe(SO_4).7H_2O$: 9.95 x 10^{-4} g L^{-1}; KH_2PO_4: 1.43 x 10^{-5} g L^{-1}; $Na_2EDTA.2H_2O$: 2.5 x 10^{-3} g L^{-1}.

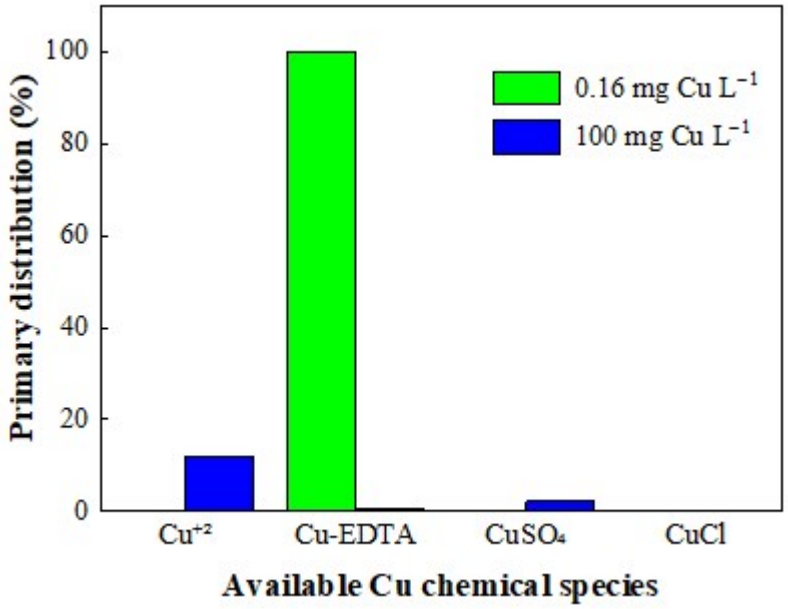

Fig. S4. Cu chemical interactions in the daphnid culture medium and after adding different concentrations of $CuSO_4$.

For the 0.16 mg Cu L^{-1} (LC_{50} $CuSO_4$), pH 7.4:
0.02% in complexed with OH^-
99.98% complexed with EDTA

For 100 mg Cu L^{-1} solution of $CuSO_4$ at pH 5.8:
12.04% as free metal
2.25% complexed with SO_4^{2-}
85.02% in solid form with OH^-
0.19% complexed with OH^-
0.08% complexed with Cl^-
0.42% complexed with EDTA

Sensitivity assay

Sensitivity assays were performed with the reference substance NaCl before acute and chronic assays. Neonates (≤ 24 hold) were exposed to concentrations of 1, 3, 5 and 7 g L^{-1} with five neonates (≤ 24 hold) per replicate and the resulting dose-response curve for the effect of NaCl on daphnid survival is shown in Fig. S5.

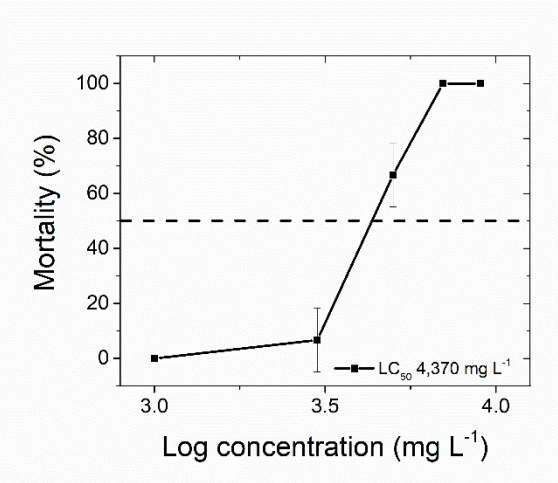

Fig. S5. Dose-response curve for the acute toxicity of the reference chemical NaCl to *Daphnia magna*.

Literature reported LC_{50} values for nCuO and soluble counterparts

Table S3 shows the LC_{50} values found by other research groups for the acute toxicity of CuO nanoparticles to *D. magna*.

Table S3. LC_{50} values found in the present study and in the literature for *Daphnia magna* exposed for 48 hours to the CuO nanoparticles, $CuSO_4$ and other Cu salts. For CuO nanoparticles the LC_{50} varied from 0.05 to 4.0 mg Cu L^{-1}, whereas for positive controls (soluble Cu forms) it ranged from 0.02 to 0.80 mg Cu L^{-1}.

Reference	nCuO (nm)	LC_{50} nCuO (mg Cu L^{-1})	LC_{50} (mg Cu L^{-1})
2	< 50	4.0	$CuCL_2$ – 0.8
3	30	4.0	-
4	30	3.2	$CuSO_4$ – 0.17
5	200-300	22	$CuSO_4$ – 0.10
6	< 50	2.79	$CuCl_2$ – LC_{50} not report
7	30-50	1.09	$Cu(NO_3)_2$ 0.02
8	30-50	0.98	$CuSO_4$ – 0.04
9	6	0.08	-
10	50	0.102	$Cu(NO_3)_2$ - 0.02
11	78	0.79	-
12	< 100	0.63	-
In this study		0.05	$CuSO_4$ - 0.16
In this study		2.34	same as above
In this study		2.26	same as above

Survival decrease x concentration

Fig. S6 shows the daphnid survival decrease as a function of concentration for 80 nm and 40 nm nCuO. The data used here is the same as shown in Figure 3A.

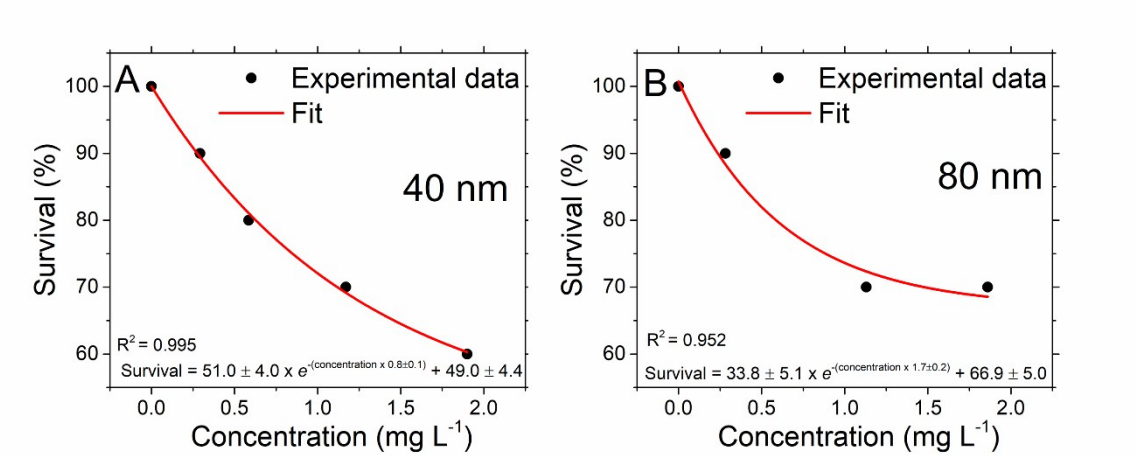

Fig. S6. Effect of (A) 40 nm and (B) 80 nm nCuO on the survival of *Daphnia magna* in chronic tests.

Statistical graphs of average of reproduction and average of neonates

Fig. S7 shows the results of the statistical analysis of (A) the average reproduction and (B) the average number of neonates. The lowest average reproduction was obtained for $CuSO_4$ and 25 nm nCuO, especially at 0.00064 mg Cu L^{-1} and 0.01248 mg Cu L^{-1}, respectively. Analogous to this, the lowest average number of neonates was found for $CuSO_4$ and 25 nm nCuO.

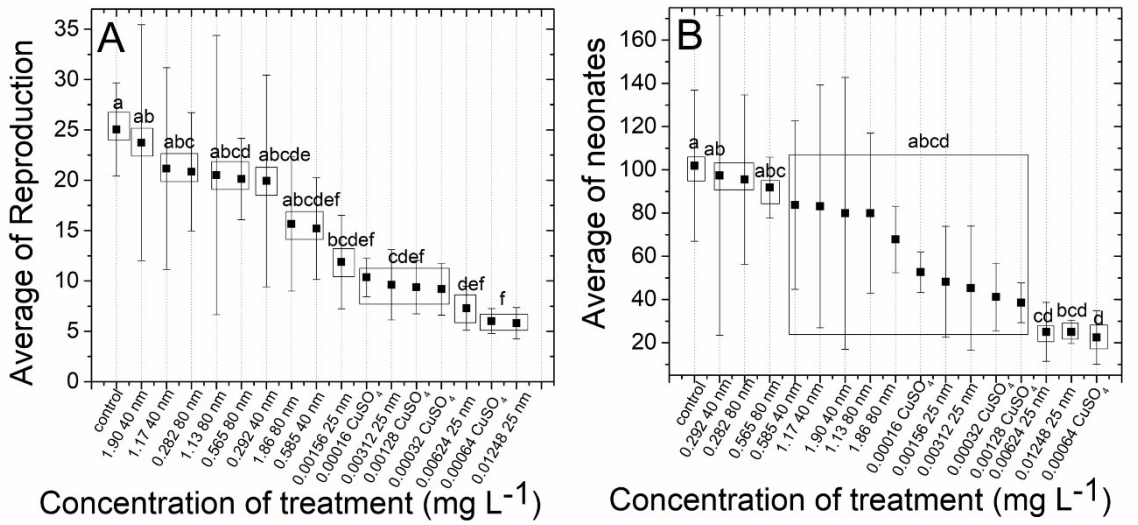

Fig. S7. (A) Average reproduction and (B) average number of neonates produced by *Daphnia magna* in 21-day chronic toxicity assays. Values followed with the same indices do not differ significantly according to the Tukey test ($p < 0.05$).

Chemical reactivity (H_2O_2 decomposition)

Fig. S8 shows the O_2 production curves from H_2O_2 decomposition in function of time at 1,000 mg Cu L^{-1} in (A) deionized water, (B) culture medium and (C) at the concentration corresponding with the LC_{50} for effects on the daphnids in culture medium.

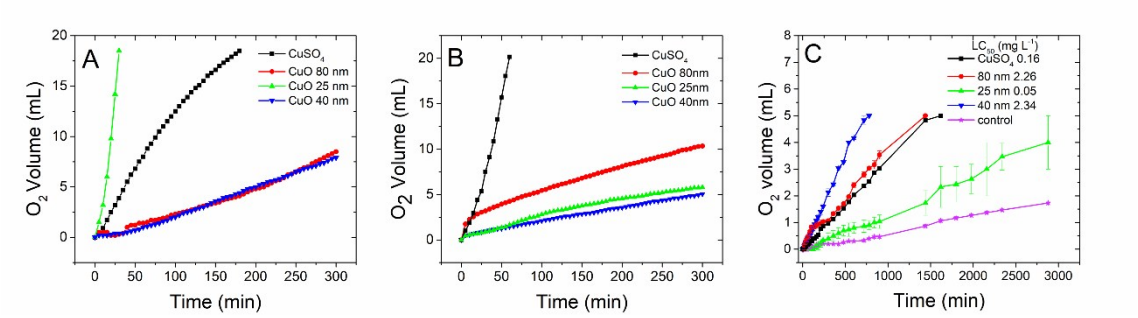

Fig. S8. Oxygen volume generated during the decomposition of H_2O_2 in the presence of nCuO and $CuSO_4$ at 1,000 mg Cu L^{-1} in (A) deionized water, (B) daphnid culture medium and (C) at concentrations in daphnid culture medium corresponding with the LC_{50}s for the effects of these compounds on the survival of *Daphnia magna*.

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