

Supporting Information

Trophic transfer and accumulation of multiwalled carbon nanotubes in the presence of copper ions in *Daphnia magna* and fathead minnow (*Pimephales promelas*)

*Amanda M. Cano*¹, *Jonathan D. Maul*¹, *Mohammad Saed*², *Fahmida Irin*³, *Smit A. Shah*⁴, *Micah J. Green*⁴, *Amanda D. French*¹, *David M. Klein*¹, *Jordan Crago*¹, *Jaclyn E. Cañas-Carrell*¹

¹Department of Environmental Toxicology, The Institute of Environmental and Human Health, Texas Tech University, Lubbock, Texas United States

²Department of Electrical and Computer Engineering, Texas Tech University, Lubbock, Texas United States

³Department of Chemical Engineering, Texas Tech University, Lubbock, Texas United States

⁴Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, Texas United States

Number of Pages: S1-S9

Number of Figures: 1-6

Number of Tables: 1

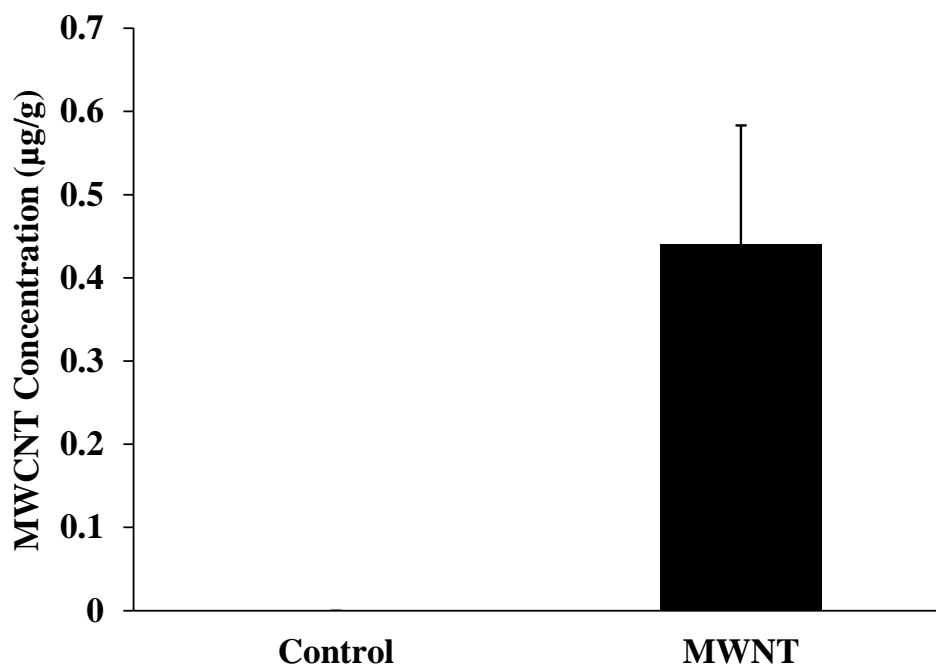


Figure S1. Preliminary study of fathead minnow (FHM, n=5 per treatment) exposed for 7 d to MWCNTs (0.1 mg/L). FHM were fed *D. magna* previously exposed to MWCNTs (0.1 mg/L) for 7 d. Treatments included a control and MWCNTs with 8–15 nm OD. There was a significant difference between control and FHM exposed to MWCNT1 ($p < 0.05$). Significant differences were compared between treatments using nonparametric statistical tests. No MWCNTs were detected in control treatments.

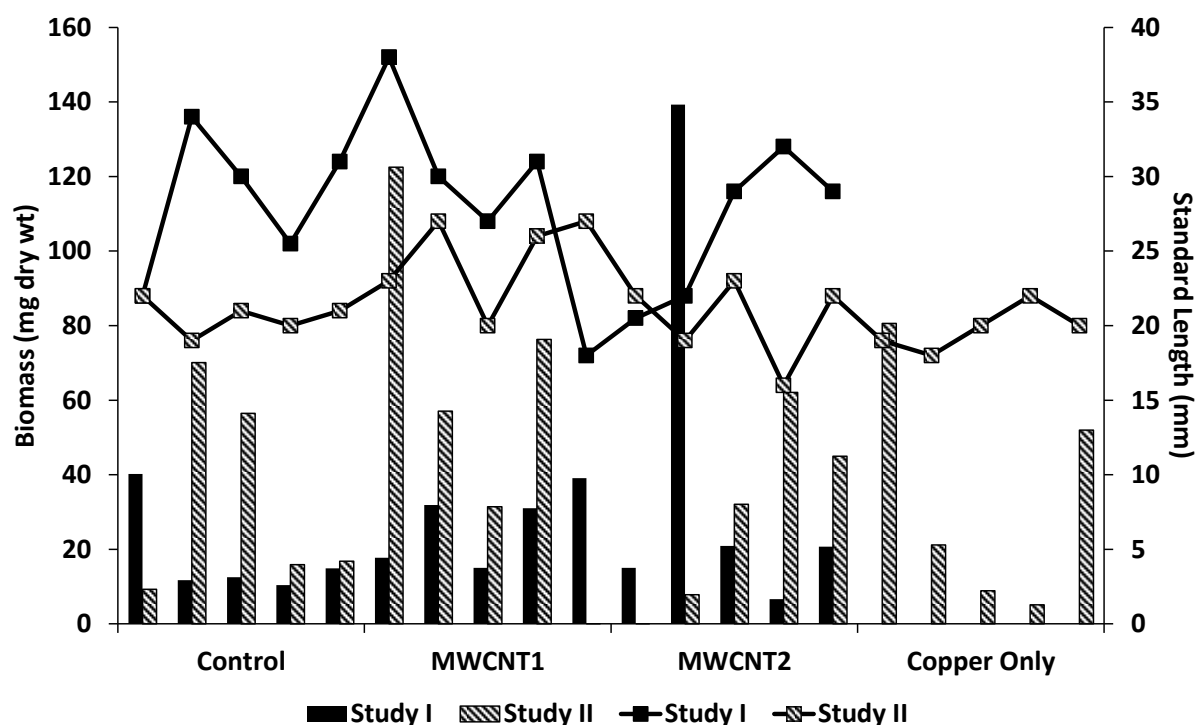


Figure S2. Dry biomass and standard length of whole fathead minnow (FHM) after 7 d trophic transfer studies with study I: FHM (n=15) fed *D. magna* exposed to MWCNT only (0.1 mg/L) and study II: FHM (n=20) fed *D. magna* exposed to MWCNT (0.1 mg/L) and Cu^{2+} (0.01 mg/L). Shown here is the dry mass of fish for control, copper only, MWCNTs with 8–15 nm OD (MWCNT1), and MWCNTs with 20–30 nm OD (MWCNT2) treatments. Biomass is represented as the primary axis, standard length is represented by the secondary axis.

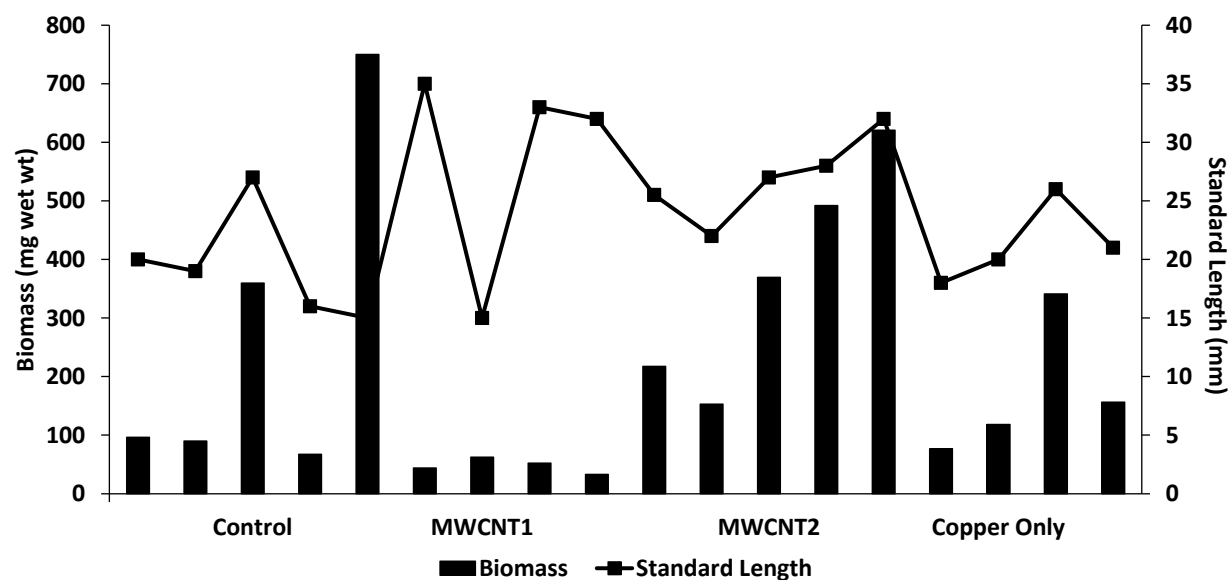


Figure S3. Wet biomass and standard length of whole fathead minnow (FHM) after 7 d trophic transfer studies with study III: FHM (n=20) fed *D. magna* exposed to MWCNT (0.1 mg/L) and Cu^{2+} (0.01 mg/L). Shown here is the wet mass of fish for control, copper only, MWCNTs with 8–15 nm OD (MWCNT1), and MWCNTs with 20–30 nm OD (MWCNT2) treatments. Biomass is represented as the primary axis, standard length is represented by the secondary axis.

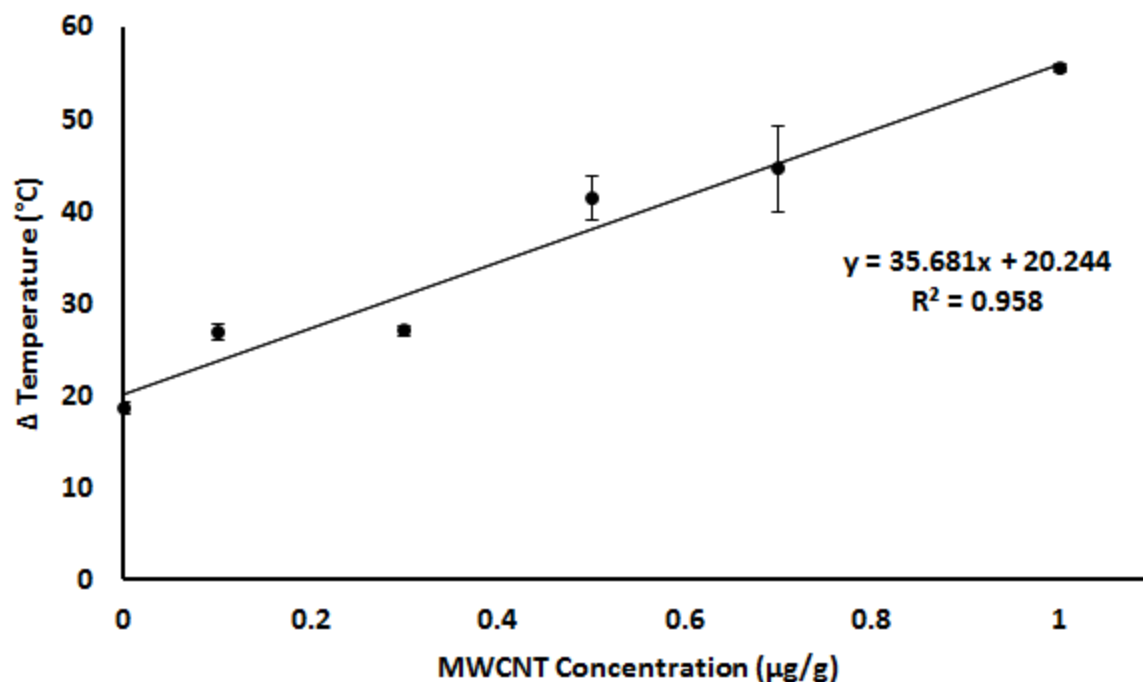


Figure S4. Calibration curve for MWCNT measurements in fathead minnow using microwave-induced heating. Samples were exposed to 20 W of microwave power for 10 s. Each point was measured 3 times, error bars show standard error between measurements.

Table S1. Copper concentration in *D. magna* and fathead minnow diets during tank holding period and studies.

	Copper Concentration (mg/L)
Brine Shrimp	$0.03 \pm 2.0 \times 10^{-4}$
YCT	$0.49 \pm 7.0 \times 10^{-3}$
Green Algae	0.50 ± 0.03

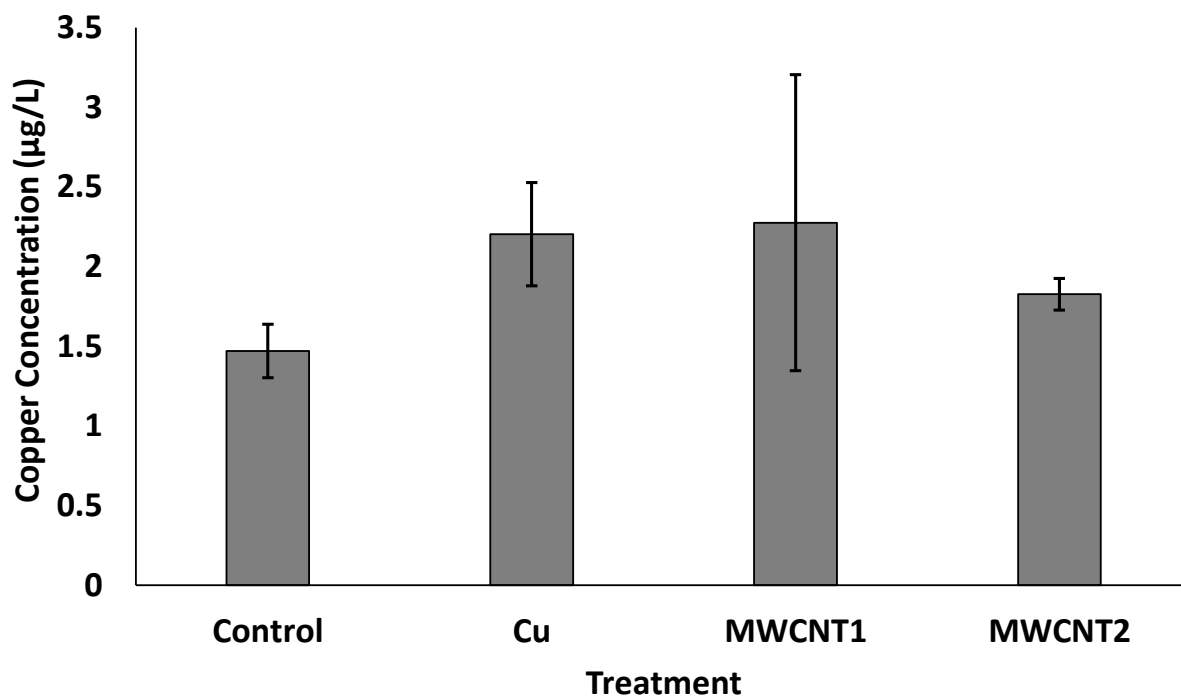


Figure S5. Copper ion concentration measured in tank water during study II treatments ($n = 5$) which housed fathead minnow fed *D. magna* exposed to MWCNTs (0.1 mg/L) and copper (0.01 mg/L) concentrations. Treatments represent control, copper only (Cu), MWCNT with 8–15 nm OD (MWCNT1), and MWCNTs with 20–30 nm OD (MWCNT2). Error bars represent standard error.

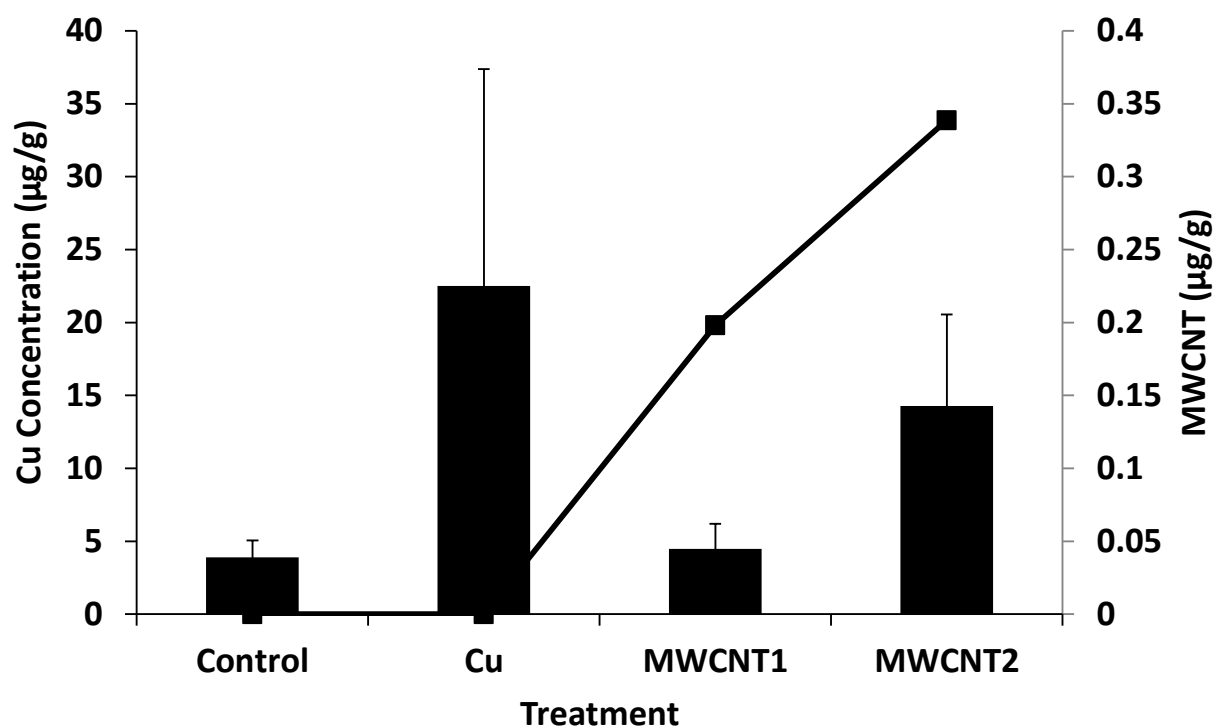


Figure S6. Comparison of copper ion and MWCNT concentrations in fathead minnow (n=5) fed *D. magna* exposed to MWCNTs (0.1 mg/L) and copper (0.01 mg/L) concentrations. Treatments represent control, copper only (Cu), MWCNT with 8–15 nm OD (MWCNT1), and MWCNTs with 20–30 nm OD (MWCNT2). Error bars represent standard error.

MWCNT calibration curve preparation

MWCNTs were measured using a microwave-induced heating technique which has been used in other biological matrices.¹⁻⁴ A calibration curve was constructed for both *D. magna*⁴ and FHM matrices. For the FHM curve, ten control fish from the holding tank were sacrificed. Each calibration point for FHM was baked at 70 °C for 2 d. FHM samples were grounded into a powder and a precision pipette was used to spike known concentrations of 0, 0.1, 0.3, 0.5, 0.7, and 1.0 µg/g (wt/dry wt) from MWCNT dispersions. Samples were then baked for an additional 2 d at 70 °C. After drying, each sample was placed into a glass vial (4 cm I.D., 6.5 cm long). Each calibration point had a consistent dry mass (10–15 mg) to prevent variability. These points were measured using a microwave-induced heating method described in previous studies.¹ Briefly, the difference between the initial and final temperature was measured using a K-type thermocouple (Model SC-GG-K-30-36, ungrounded, 0.081 cm diameter, Omega Engineering Inc., Stamford, CT) connected to a multi-meter (Model HHM209, Omega Engineering Inc., Stamford, CT) to construct a FHM calibration curve.

References Cited

1. Irin, F.; Shrestha, B.; Canas, J. E.; Saed, M. A.; Green, M. J. Detection of carbon nanotubes in biological samples through microwave-induced heating. *Carbon* **2012**, *50* (12), 4441–4449.
2. Li, S. B.; Irin, F.; Atore, F. O.; Green, M. J.; Canas-Carrell, J. E. Determination of multi-walled carbon nanotube bioaccumulation in earthworms measured by a microwave-based detection technique. *Sci. Total Environ.* **2013**, *445*, 9–13.
3. Cano, A. M.; Kohl, K.; Deleon, S.; Payton, P.; Irin, F.; Saed, M.; Shah, S. A.; Green, M. J.; Cañas-Carrell, J. E. Determination of uptake, accumulation, and stress effects in corn (*Zea mays* L.) grown in single-wall carbon nanotube contaminated soil. *Chemosphere* **2016**, *152*, 117–122.
4. Cano, A. M.; Maul, J. D.; Saed, M.; Shah, S. A.; Green, M. J.; Cañas-Carrell, J. E. Bioaccumulation, stress, and swimming impairment in *Daphnia magna* exposed to multiwalled carbon nanotubes, graphene, and graphene oxide. *Environ. Toxicol. Chem.* **2017**, *36* (8), 2199–2204.