SUPPORTING INFORMATION

Metal-polycyclic aromatic hydrocarbon mixture toxicity in *Hyalella azteca*. II. Metal accumulation and oxidative stress as interactive co-toxic mechanisms

Patrick T. Gauthier[†], *Warren P. Norwood*[‡], *Ellie E. Prepas*[†], *Greg G. Pyle*[*§]

[†]Faculty of Natural Resources Management, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1
[‡]Aquatic Contaminants Research Division, Environment Canada, Burlington, Ontario, Canada L7S 1A1
[§]Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4
[*]Corresponding author. Tel: +1 (403) 332-4048. E-mail address: gregory.pyle@uleth.ca

Summary of supporting information

Figure S1. Tissue Cd concentrations and lethal response of juvenile *H. azteca* in Cd-only exposures (i.e., weekly references tests) from Cd-phenanthrene (PHE) and Cd-phenanthrenequinone (PHQ) isobole experiments. Closed circles with standard errors represent LBC50 estimates for each curve. S2

Figure S2. Redox-cycling of phenanthrenequinone and Cu.[1-4] 'SOD' denotes superoxide dismutase. 'oxR' denotes NADH:ubiquinone oxidoreductase. 'DTD' denotes NAD(P)H:quinone oxidoreductase S3

Table S1. Measured concentrations of metals and polycyclic aromatic hydrocarbons (PAHs) from metal accumulation assays. 'p_{ri}' denotes the mixture proportions based on equitoxic concentrations, and represents the proportion of PAH in the mixture. Thus, a $p_{ri} = 0.5$ indicates that both contaminants are present at equitoxic concentrations..... S4

References S5

pp S1 – S5

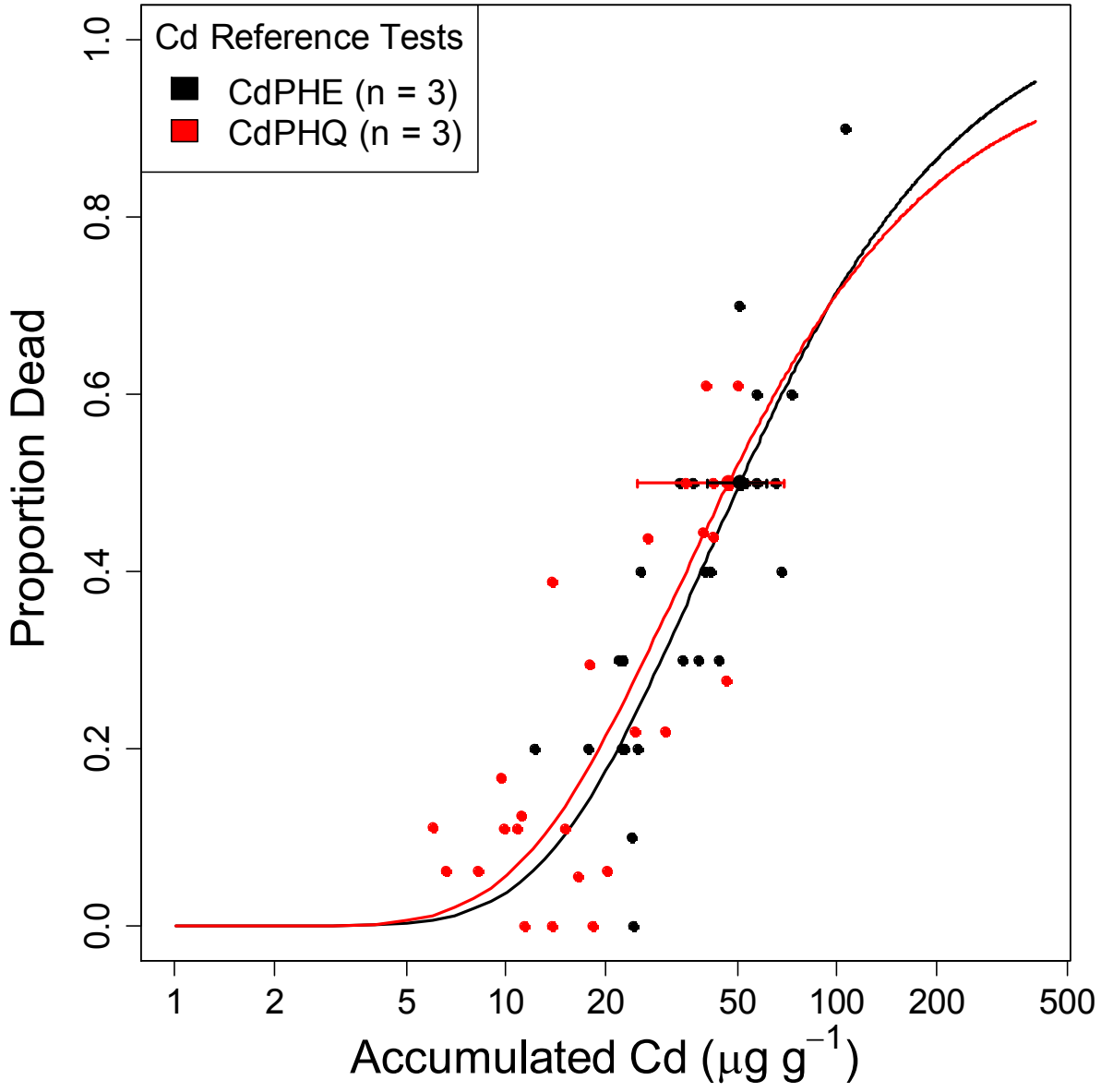

Figure S1. Tissue Cd concentrations and lethal response of juvenile *H. azteca* in Cd-only exposures (i.e., weekly references tests) from Cd-phenanthrene (PHE) and Cd-phenanthrenequinone (PHQ) isobole experiments. Closed circles with standard errors represent LBC50 estimates for each curve.

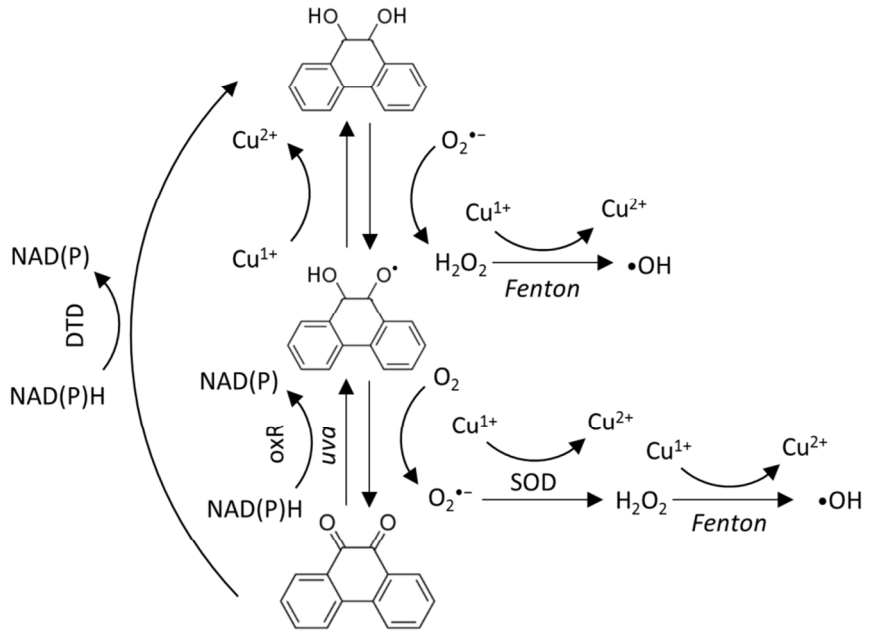

Figure S2. Redox-cycling of phenanthrenequinone and Cu.[1-4] 'SOD' denotes superoxide dismutase. 'oxR' denotes NADH:ubiquinone oxidoreductase. 'DTD' denotes NAD(P)H:quinone oxidoreductase.

Table S1. Measured concentrations of metals and polycyclic aromatic hydrocarbons (PAHs) from metal accumulation assays. 'p_{ri}' denotes the mixture proportions based on equitoxic concentrations, and represents the proportion of PAH in the mixture. Thus, a p_{ri} = 0.5 indicates that both contaminants are present at equitoxic concentrations.

mixture	p_{ri}	metal concentration (μg L^{-1})	PAH concentration (μg L^{-1})
Cu-PHE	0.86	2.5	77.7
	0.86	4.3	141.0
	0.86	7.0	221.3
	0.56	6.4	61.4
	0.56	12.2	86.3
	0.30	15.6	24.3
Cu-PHQ	0.82	2.2	240.9
	0.82	3.6	499.4
	0.82	5.3	765.4
	0.57	3.8	144.1
	0.57	6.8	321.3
	0.27	7.3	51.2
Cd-PHE	0.62	5.5	87.9
	0.62	9.3	192.0
	0.62	13.0	317.1
	0.36	10.6	61.3
	0.36	20.2	146.6
	0.36	28.8	248.2
	0.16	14.9	28.9
	0.16	25.9	73.7
	0.16	38.8	145.0
Cd-PHQ	0.59	1.8	42.9
	0.59	3.9	95.2
	0.59	11.1	324.3
	0.45	4.1	35.4
	0.45	6.9	53.6
	0.45	22.7	169.7
	0.12	5.4	1.3
	0.12	11.9	32.9
	0.12	35.9	95.7

References

(1) Flowers-Geary, L.; Harvey, R.; Penning, T. Cytotoxicity of polycyclic aromatic hydrocarbon *o*-quinones in rat and human hepatoma cells. *Chem. Res. Toxicol*. **1993**, *6* (3), 252-260; DOI: 10.1021/tx00033a002.

(2) Penning, T.; Ohnishi, S.; Ohnishi, T.; Harvey, R. Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon trans-dihydrodiols catalyzed by dihydrodiol dehydrogenase. *Chem. Res. Toxicol*. **1996**, *9* (1), 84-92; DOI: 10.1021/tx950055s.

(3) Xie, F.; Koziar, S. A.; Lampi, M. A.; Dixon, D. G.; Norwood, W. P.; Borgmann, U.; Huang, X.; Greenberg, B. G. Assessment of the toxicity of mixtures of copper and 9,10-phenanthrenequinone, and phenanthrene to *Daphnia magna*: evidence for a reactive oxygen mechanism. *Environ. Toxicol. Chem.* **2006**, *25* (2), 613-622; DOI: 10.1897/05-256R.1.

(4) Wang, W.; Nykamp, J.; Huang, X.; Gerhardt, K.; Dixon, D. G.; Greenberg, B. M. Examination of the mechanism of phenanthrenequinone toxicity to *Vibrio fischeri*: evidence for a reactive oxygen species-mediated toxicity mechanism. *Environ. Toxicol. Chem.* **2009**, *28* (8), 1655-1662; DOI: 10.1897/08-463.1.