Environ. Chem. **2016**, *13*, 447–456
doi:10.1071/EN15124 _AC

Supplementary material

Evaluating the concentration addition approach for describing expected toxicity of a ternary metal mixture (Ni, Cu, Cd) using metal speciation and response surface regression

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Table S1. Measured total metal concentrations ([M_{Tot}]) *v.* WHAM 7-predicted dissolved and free concentrations of Ni, Cu, Cd in single-metal exposures in APHA media (pH 8.3), and expressed as portion (%) of total measured concentration in solution

Test sample	Measured [M_{Tot}] (μg L^{-1})	Predicted [M_{Diss}] (μg L^{-1})	Dissolved (percentage of total)	Predicted {M^{2+}} (μg L^{-1})	Free (percentage of total)
Ni 0	0	0	87	0.1	38
Ni 1	1	1	89	0.4	35
Ni 2	2	2	91	0.8	37
Ni 3	4	4	92	1.5	38
Ni 4	8	7	94	3.1	40
Ni 5	13	12	95	5.3	42
Ni 6	34	33	97	14.2	42
Ni 7	86	84	98	40.4	47
Cu 0	4	3	71	0.03	1
Cu 1	5	3	72	0.03	1
Cu 2	10	8	80	0.07	1
Cu 3	12	10	82	0.08	1
Cu 4	22	19	86	0.17	1
Cu 5	34	30	89	0.28	1
Cu 6	58	53	91	0.56	1
Cu 7	119	112	94	1.59	1
Cd 0	0	0	–	0.0	43
Cd 1	1	1	99	0.3	43
Cd 2	2	2	99	0.8	44
Cd 3	3	3	99	1.5	45
Cd 4	12	12	99	5.6	44
Cd 5	20	20	99	8.9	44
Cd 6	45	44	99	19.7	47
Cd 7	88	87	99	41.5	43

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Table S2. Copper hydroxide and carbonate speciation (as a percentage of total) in the single-metal and mixture test exposures in APHA media (pH 8.3), as predicted by WHAM 7

Test sample	$CuOH^+$	$Cu(OH)_2$	$CuHCO_3^+$	$CuCO_3$
Cu 0	4	2	19	54
Cu 1	4	2	18	56
Cu 2	4	2	21	58
Cu 3	4	2	18	55
Cu 4	4	2	21	58
Cu 5	4	2	21	56
Cu 6	4	2	21	59
Cu 7	4	2	23	57
Mix 1	4	2	20	59
Mix 2	4	2	20	60
Mix 3	4	2	17	51
Mix 4	4	2	22	59
Mix 5	4	2	21	58
Mix 6	4	2	21	59
Mix 7	4	2	21	58
Mix 8	4	2	23	57
Mix 9	4	2	22	58
Mix 10	4	2	22	58
Mix 11	4	1	22	55
Mix 12	4	1	22	55
Mix 13	4	2	19	54
Mix 14	4	2	18	56
Mix 15	4	2	21	58
Mix 16	4	2	18	55
Mix 17	4	2	21	58
Mix 18	4	2	21	56
Mix 19	4	2	21	59
Mix 20	4	2	23	57

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Table S3. Full range of toxicity thresholds, from statistical no-effect concentration (SNEC) to EC_{90}, for single-metal exposures of Ni, Cd and Cu as total metal concentration or free-ion activity (as predicted by WHAM 7), and when endpoint was root length growth or frond count

All thresholds are in units of microgram per litre, and the 5–95 % confidence interval is presented in the parentheses, and N/A (not applicable) is listed when the lower or upper limit was not calculable

		Root length		Frond count	
		Total	Free	Total	Free
Ni	SNEC	12.1 (6.9–17.4)	4.89 (2.71–7.13)	4.52 (1.96–7.36)	1.68 (0.69–2.83)
	EC_{10}	6.9 (1.8–10.8)	2.74 (0.64–4.35)	5.03 (2.31–8.05)	1.90 (0.83–3.13)
	EC_{25}	13.4 (8.1–19.0)	5.43 (3.22–7.82)	14.8 (9.7–20.8)	6.01 (3.86–8.65)
	EC_{50}	25.7 (18.7–35.5)	10.8 (7.7–15.1)	43.4 (31.7–59.3)	19.0 (13.6–26.6)
	EC_{60}	32.8 (23.6–47.5)	13.9 (9.8–20.6)	64.5 (44.7–96.0)	29.1 (19.6–44.6)
	EC_{75}	49.6 (33.5–90.3)	21.3 (14.0–40.9)	127 (77–253)	60.1 (34.9–126.0)
	EC_{80}	58.8 (38.6–129)	25.5 (16.2–60.2)	169 (96–406)	81.2 (44.4–209.1)
	EC_{90}	95.4 (57.4–N/A)	42.3 (24.6–N/A)	374 (184–2844)	190 (89–1692)
Cu	SNEC	13.9 (7.8–19.4)	0.0675 (0.0305–0.1066)	6.28 (2.49–10.77)	0.0321 (0.0091–0.0670)
	EC_{10}	15.0 (8.8–20.7)	0.0855 (0.0456–0.1283)	6.60 (2.83–11.03)	0.0303 (0.0075–0.0656)
	EC_{25}	31.5 (23.5–40.1)	0.239 (0.165–0.326)	21.6 (13.9–31.2)	0.150 (0.085–0.242)
	EC_{50}	66.2 (53.5–81.8)	0.670 (0.505–0.889)	70.7 (50.0–99.8)	0.743 (0.464–1.189)
	EC_{60}	87.0 (67.9–114)	0.979 (0.702–1.402)	109 (69–183)	1.34 (0.71–2.69)
	EC_{75}	139 (98–222)	1.88 (1.18–3.51)	231 (111–726)	3.68 (1.35–17.91)
	EC_{80}	169 (115–308)	2.46 (1.46–5.47)	315 (138–1716)	5.60 (1.79–60.64)
	EC_{90}	292 (179–1219)	5.25 (2.70–32.84)	757 (264–N/A)	18.2 (4.3–N/A)
Cd	SNEC	11.7 (7.4–15.3)	4.86 (3.14–6.27)	0.294 (0.060–0.727)	0.142 (0.029–0.350)
	EC_{10}	10.9 (6.6–14.4)	4.99 (3.27–6.41)	0.759 (0.311–1.448)	0.356 (0.143–0.688)
	EC_{25}	19.4 (15.1–23.7)	8.73 (7.05–10.43)	6.10 (4.19–8.59)	2.87 (1.95–4.07)
	EC_{50}	34.3 (29.4–40.2)	15.3 (13.2–17.7)	49.1 (35.1–68.7)	23.1 (16.4–32.5)
	EC_{60}	42.4 (36.2–50.3)	18.8 (16.1–22.1)	106 (68–169)	49.8 (31.6–80.2)
	EC_{75}	60.9 (50.0–77.9)	26.8 (22.1–33.9)	395 (205–875)	186 (94–423)
	EC_{80}	70.8 (56.9–95.1)	31.0 (25.1–41.2)	681 (324–1807)	320 (149–880)
	EC_{90}	108 (82–180)	46.9 (35.9–75.4)	3172 (1211–16760)	1493 (556–8530)

doi:10.1071/EN15124 _AC

Table S4. Concentration (dose) addition (CA) for each test case when Cu is solved for and *Y* = 50 % RGI

Concentrations and free ion activities of Cd and Ni are held at 1/3 of their original test case value. C_i is the concentration of the metal in the mixture causing 50 % RGI, and ΣTU is the sum of C_i divided by $EC50_i$ from the single-metal toxicity studies

Test Sample	Total metal				Free-ion activity			
	C_i			ΣTU	C_i			ΣTU
	Ni = 1/3	Cu = *x*	Cd = 1/3		Ni = 1/3	Cu = *x*	Cd = 1/3	
1	6.58	34.60	7.53	1.00	8.04	0.11	9.92	0.83
2	24.23	13.40	6.50	1.33	29.29	0.10	8.44	1.20
3	6.30	36.50	6.37	0.98	7.84	0.40	8.46	0.81
4	6.31	11.90	28.03	1.25	7.58	0.10	36.48	1.16
5	25.28	11.00	6.87	1.34	31.12	0.36	9.04	1.22
6	26.47	–11.80	27.13	1.64	33.06	0.22	36.16	1.60
7	6.54	13.90	25.67	1.22	8.11	0.60	33.98	1.14
8	26.35	–8.97	23.43	1.57	33.67	0.59	31.57	1.54
9	0.26	30.60	15.93	0.94	0.31	0.41	20.91	0.80
10	31.70	–11.00	15.80	1.52	38.45	0.40	20.60	1.44
11	16.13	12.40	16.17	1.28	19.35	0.03	21.01	1.18
12	15.94	13.20	15.67	1.27	19.92	0.81	20.82	1.19
13	16.58	35.00	0.57	1.18	20.65	0.40	0.75	1.00
14	17.84	–5.51	33.37	1.58	22.01	0.43	43.97	1.54
15	16.41	11.30	16.77	1.30	20.35	0.41	22.17	1.21
16	15.76	13.00	16.10	1.28	19.94	0.44	21.64	1.20
17	16.10	12.90	15.77	1.28	20.18	0.43	21.03	1.19
18	16.14	12.80	15.83	1.28	20.22	0.41	21.13	1.19
19	14.45	23.00	9.53	1.18	18.33	0.23	12.82	1.07
20	9.99	32.10	6.83	1.07	12.68	0.24	9.19	0.92
Average				1.27				1.17