

Supporting Information

Gene Expression Profiling in *Daphnia magna* Part 1: Concentration Dependent Profiles Provide Support for a No Observed Transcriptional Effect Level

Helen C. Poynton, Alexandre V. Loguinov, Julia R. Varshavsky, Sarah Chan,
Edward J. Perkins and Chris D. Vulpe

Table S1: Description of exposure levels for microarray hybridizations

Exposure level	Description
1/20 EC ₅₀	1/20th of the concentration that causes a 50% reduction in reproduction during a 21-d, chronic exposure
1/10 EC ₅₀	1/10th of the concentration that causes a 50% reduction in reproduction during a 21-d, chronic exposure
1/10 LC ₅₀	1/10th of the concentration that causes a 50% reduction in survival during a 24-h, acute exposure
NOEC	The highest concentration that does not cause a significant ($p > 0.05$) reduction in survival after a 24-h acute exposure

Exposure levels were chosen based on acute (24-h) and chronic (21-d) toxicity endpoints. These levels were chosen to represent a tolerated concentration (1/20 EC₅₀ or 1/10 EC₅₀), a sublethal concentration (1/10 LC₅₀), and a nearly acutely toxic concentration (NOEC).

Table S2: Primer sequences used for *Real-time* qPCR confirmation of microarray data

Accession Number	Predicted Function	Primer Sequences
DV437798	Monooxygenase	CCATGTCGACGTCTTTCTGA AGGCCTGATGACAACGTCTT
DV437830	Glutathione-S-transferase (GST)	TCAGGCTGGTGTGAGTTTG GAGCAAGCATTGTCCATCA
DV437806	Inositol monophosphatase (IMPase)	CATGCAGGCAACATAATTCTG CTGTTGAGCTCTGCGATCAG
DV437797	Cellulase	TTCCAGTTTCCAGGTTGACA GGACGTAGAAACGACGGAAT
ES408209	Preamylase	CGACATTATCTCGGGCAACT AGGCCTTTCATCAGATCCT
DV437805	Slit homolog	GTCGATTTTTTCGGTCCTCAA AGGTCAGGCAGTGTTCGAT
DV437826	Metallothionien (MT a)	TTGCCAAAACAATTGCTCAT CACCTCCAGTGGCACAAAT
DV437799	Metallothionien (MT b)	GTGGAACCGAATGCAAATG TGCATGGACAACTGGAACGT
DV437813	Lectin	TTTCGTGCTACTGCGCTACT GCTCCTTCTCGTACGTTTCC
DV437823	β -1,3-glucan binding protein (BGBP)	ATCGCTACCAACTGGAATGG GACGTACCGAGTTCCAGAA
DV437858	Chitinase	CGAAACCACGTTCAAGATCA CAAGCCGGTGAATTTACGAT
AF070104	18S rRNA	TGTATCCCAACGCCTGAATA CCGAGGTCCTATTCCATCAT

Primer pairs for *real time* PCR were designed using Primers3 software (1) according to the recommendations of Applied Biosystems for the SYBR GREEN PCR based assay. (Source code available for Primers3 at <http://fokker.wi.mit.edu/primer3/>)

Table S3: Candidate Differentially Expressed Genes

PROTEIN HOMOLOG	NCBI Acc#	1/20EC50 Cu	1/10EC50 Cu	1/10LC50 Cu	NOEC Cu	1/10 EC50 Cd	1/10LC50 Cd	NOEC Cd	1/10 EC50 Zn	1/10LC50 Zn	NOEC Zn
Digestion											
Q95YH1: Cellulase	DV437797	-0.296	-1.134	-1.422	-2.475	-0.129	-1.380	-2.056	0.314	-1.110	-2.381
AAO17927: α -amylase	DV437794	-0.185	0.918	-0.881	-1.290	0.026	0.972	-0.250	0.334	-1.120	-0.550
Q26193: Preamylase 1 pre.	DV437815	-0.169	-0.503	-0.656	-0.979	0.195	-0.333	0.212	0.209	-1.213	-0.802
QRWPI2: Endo- β -1,4-mannanase	DV437795	-0.219	-0.977	-1.016	-0.949	-0.012	-0.890	-0.923	0.378	-0.848	-1.712
XM_792007: Hydrolase	ES408267	-0.071	-0.581	-0.309	-0.903	-0.102	-0.253	-0.194	0.211	-0.156	-0.884
Q9BH22: Endo- β -1,4-glucanase	DV437796	-0.080	-0.519	-1.009	-0.638	-0.057	-0.586	-0.533	0.404	-0.775	-1.071
AY081706: Cellobiohydrolase I	ES408211	-0.020	-0.393	-0.120	-0.532	-0.033	-0.141	-0.534	0.120	-0.110	-0.483
Q9TU53: Cubilin	DV437819	-0.020	-0.151	-0.435	-0.517	0.212	-0.184	-0.597	-0.020	0.236	-0.150
Peptidases											
AAH64208: Trypsin II precursor	DV437844	-0.079	0.183	-0.405	-0.482	0.094	-0.642	-0.143	0.035	-0.587	-0.008
Q9MRH5: Serine protease	DV437843	-0.014	0.266	0.316	-0.116	0.049	-0.636	-0.007	-0.011	-0.213	0.242
Q9VZ49: Serine protease	DV437842	-0.102	-0.565	-0.356	-0.417	-0.052	-0.578	-0.905	0.159	-0.493	0.068
Q27761: Trypsin precursor	DV437836	-0.200	-0.083	0.123	-1.100	0.028	-0.554	-0.726	0.250	-0.381	-1.072
Q46137: Chymotrypsin precursor	DV437834	-0.049	0.461	-0.199	-0.854	0.065	-0.546	0.015	0.088	-0.797	0.231
Q8I0P2: Trypsin	DV437840	-0.161	-0.194	-0.250	-1.026	-0.023	-0.469	-0.942	0.130	0.290	-0.625
Q7QWQ3: Serine protease	DV437839	0.033	0.170	0.060	-0.428	0.032	-0.484	-0.251	0.086	-0.146	-0.043
AF486488: Trypsin	ES408193	0.211	0.119	0.124	0.493	0.029	0.483	0.821	0.294	0.212	1.118
Q27824: Serine collagenase 4	DV437841	-0.333	-0.203	-0.195	-1.208	-0.090	-0.344	-0.905	0.173	0.397	-1.185
P36178: Chymotrypsin BII pre.	DV437803	-0.309	-0.030	-0.176	-1.113	-0.070	-0.332	-0.627	0.167	0.676	-1.097
BX050019: Carboxypeptidase	ES408205	-0.659	-	-0.202	-1.152	0.221	-0.369	-1.092	0.305	0.416	-1.221
Q8I8P2: Trypsin	DV437853	-0.132	0.282	0.255	0.064	-0.044	-0.022	-0.238	0.115	0.808	-0.131
Q9V3M3: Zn metallopeptidase	DV437822	-0.165	-0.502	-0.675	-1.328	-0.126	0.017	-0.381	0.258	0.434	-1.303
Q9N516: Aminopeptidase	DV437812	0.061	0.161	0.399	0.643	-0.088	0.021	0.574	0.090	-0.150	-0.580
NM_001002217: Carboxypeptidase	DV437854	-0.230	0.193	-0.011	-0.572	0.105	0.058	-0.296	0.200	0.759	-1.075
AY795054: Endopeptidase	ES408379	-0.086	0.046	0.340	1.414	0.172	0.146	0.984	0.012	-0.090	1.119
Metal binding and transport											
Q9IUS3: Ferritin subunit	DV437849	-0.080	-0.187	-0.342	-0.359	0.045	-0.688	-0.625	0.096	-0.330	-0.311
AY297897: Ferritin	DV437852	0.047	-0.325	-0.279	-0.458	-0.009	-0.663	-0.718	0.038	-0.160	-0.215
Q9DUJ4: Heavy metal binding	DV437835	-0.085	-0.144	-0.334	-0.768	-0.163	-0.463	-0.196	0.051	-0.385	-0.640
AAC47544: Hemoglobin	U67067	-0.403	-0.189	0.000	-0.531	-0.574	0.000	-0.091	0.512	-0.506	0.704
NM_200198: Heme binding protein	ES408214	-0.040	0.333	0.078	1.215	0.049	0.102	0.591	0.054	-0.235	0.085
Q9NA01: Ferritin	AJ292556	-0.004	0.283	0.591	0.426	0.021	0.478	0.670	0.070	0.291	-0.039
No homology (MT)	DV437799	0.198	0.513	0.642	1.249	0.121	0.710	0.959	0.004	0.575	0.347
Q20441: Unknown (MT)	DV437826	0.064	-0.144	0.319	0.711	0.006	1.081	1.268	-0.321	0.437	0.694
Exoskeletal proteins											
AAH00393: Cuticle protein	DV437856	0.106	-0.274	0.036	-0.020	-0.087	-0.068	-0.136	-0.448	-1.247	-0.403
Q7PWC2: Chitinase	DV437857	0.126	-0.116	0.068	-0.159	0.076	0.073	0.923	0.119	-1.121	-0.953
AF026491: Chitinase	DV437850	0.090	-0.122	0.054	-0.130	0.079	0.070	0.923	0.120	-1.123	-0.915
Q7PRG6: Chitin binding	DV437800	-0.147	-0.021	0.050	-1.151	0.137	-0.610	-0.256	0.035	-0.458	-0.397
Q9V7Z2: Chitin binding	DV437850	0.149	0.072	0.225	0.602	0.078	-0.468	0.109	0.093	0.409	0.347
P91731: Chitinase	DV437809	0.131	0.228	0.431	-0.253	0.059	-0.306	-0.062	0.176	-0.393	0.000
XM_318997: Cuticle protein	ES408180	0.231	-0.092	0.076	-0.854	0.529	-0.055	0.257	0.501	-0.153	-0.036
EF203462: Cuticle protein	ES408177	-0.202	0.555	0.071	-1.191	0.814	0.106	0.294	0.411	0.047	0.131
XM_318987: Cuticle protein	ES408178	-0.029	0.446	-0.120	-1.505	0.735	0.036	0.052	0.341	0.054	0.130
XM_00119659: Cuticle	ES408165	-0.051	0.472	-0.056	-0.944	0.895	0.158	0.363	0.201	0.132	0.158
AE003563: Cuticle protein	ES408176	-0.146	0.489	-0.080	-1.745	0.694	0.035	0.029	0.297	0.192	-0.033
AY432732: Cuticle protein	ES408164	-0.242	0.911	-0.135	-1.668	1.517	0.030	0.124	0.396	0.171	0.416
Reproductive function and development											
CR688752: Placental protein	ES408215	-0.191	-0.618	-0.408	-0.313	-0.087	-0.413	-1.041	0.170	-0.591	0.062
RAD05137: Vitellinogen SOD	AB114859	-0.284	-0.523	-0.323	-0.836	-0.137	-0.701	-1.007	0.025	-0.064	-0.095
U56966: Vitellinogen	ES408219	-0.455	-0.610	-0.190	-1.527	-0.690	-0.452	-1.007	-0.158	-0.192	-0.799
AB252738: Vitellinogen-SOD2	ES408217	-0.869	-0.532	-0.218	-0.700	-0.711	-0.277	-0.912	-0.384	-0.255	-0.853
AB114859: VTG-SOD	AB114860	-0.316	-0.380	-0.232	-0.680	-0.214	-0.314	-0.735	-0.073	-0.118	-0.595
Q84440: Posterior end mark	DV437824	0.103	-0.252	-0.524	-0.520	0.455	-0.582	-0.668	-0.034	-0.401	-0.785
Q9VEY6: Hormone reg. peptidase	DV437846	0.045	0.334	0.262	-0.082	0.044	-0.640	-0.353	-0.017	-0.332	0.187
Q7Z407: CUB and sushi	DV437847	-0.099	0.346	-0.169	-0.412	-0.020	-0.335	-0.293	0.035	0.021	-0.132
Monoxygenases											
NM_000016: Acyl coA dehydrogenase	ES408245	0.070	0.988	0.049	1.894	0.848	0.053	0.367	0.325	0.084	0.366
Q95VU5: Dopa β -hydroxylase	DV437820	0.020	-0.329	-0.451	-0.548	0.439	-0.437	-0.638	-0.038	-0.485	-0.400
Q26490: Retinol dehydratase	DV437801	0.298	-0.074	1.107	1.159	0.123	0.277	0.000	0.198	-0.774	-0.740
Q8BUZ7: Monoxygenase X	DV437798	0.097	1.023	0.291	2.271	-0.003	1.519	1.760	-0.013	0.548	1.597
Immune Function											
Q7QCT4: β -1,3-glucan binding	DV437823	-0.251	-0.809	-0.756	-1.639	0.019	-0.299	-0.849	0.274	-0.287	-1.531
Q8IUN9: Macrophage lectin	DV437813	-0.147	-0.350	-0.440	-0.613	-0.017	-0.298	-0.394	-0.110	-0.161	-0.633
Oxidative Stress Response											
Q8T5Q7: Peroxiredoxin V	DV437829	0.082		0.178	0.384	-0.445	0.619	0.425	0.040	0.347	0.031
P46436: Glutathione S-transferase	DV437830	-0.038	0.051	0.108	1.060	0.074	0.482	0.727	0.023	0.187	0.011
Q9NAW7: Glutathione S-transferase	DV437833	-0.017	0.391	0.306	0.118	0.067	0.608	0.541	0.033	0.380	0.034
Cell signaling											
Q9PT14: Precerebellin-like	DV437848	-0.122	-0.127	-0.486	-1.291	-0.106	-0.747	-0.674	0.192	-0.623	-1.748
Q9VAG9: Inositol monophosphatase	DV437906	0.140	0.204	0.634	0.846	0.000	0.156	0.441	0.014	-0.497	0.570
P51150: Ras-related, Rab-7	DV437832	0.072	0.529	0.335	1.388	-0.041	0.435	0.936	0.072	0.261	0.697
XM_647306: Cell division kinase	ES408226	-0.112	0.271	0.285	1.298	0.104	0.316	0.882	-0.083	-0.018	0.552
Q7QB55: Protein kinase	DV437828	0.136	-0.150	0.162	0.746	0.098	0.634	1.216	-0.225	0.271	0.753

PROTEIN HOMOLOG	NCBI Acc#	1/20EC50 Cu	1/10EC50 Cu	1/10LC50 Cu	NOEC Cu	1/10 EC50 Cd	1/10LC50 Cd	NOEC Cd	1/10 EC50 Zn	1/10LC50 Zn	NOEC Zn
Lipid metabolism and transport											
AA864350: Sphingolipid metabolism	DV437837	-0.153	-0.140	-0.320	-1.122	-0.161	-0.500	-1.185	0.175	0.006	-1.284
Q81896: Heart fatty acid binding	DV437817	-0.161	-0.411	-0.522	-0.734	-0.033	-0.353	-0.849	-0.078	-0.266	-0.666
AA139384: Fatty acid binding	DV437804	-0.201	-0.182	-0.162	-1.348	0.078	-0.067	-0.316	0.129	-0.441	-0.547
AY270214: Fatty acid binding	ES408221	-0.370	-0.420	-0.218	-0.493	-0.484	-0.374	-0.818	-0.406	-0.262	-0.786
Other functions											
AAQ83892: Interferon γ -inducible	DV437831	0.924	0.378	0.358	0.534	0.114	0.446	0.836	0.059	0.079	0.182
AJ304447: Serine protease inhibitor	ES408189	-0.063	0.110	0.146	1.595	0.130	0.046	0.927	-0.010	-0.333	0.284
AY522647: Glutamine synthetase-like	ES408260	-0.119	0.885	-0.084	-1.716	1.011	0.089	0.401	0.272	0.011	0.364
NM_137626: Guanine nucleotide exchar	ES408233	-0.094	0.827	0.014	-1.482	0.908	0.141	0.471	0.203	0.091	0.368
NM_199989: Aspartate aminotransferase	ES108259	0.176	0.227	0.097	0.747	0.027	0.348	0.708	0.009	0.126	0.776
AY642708: NADH dehydrogenase	ES408243	0.072	0.501	0.010	-0.802	0.012	0.100	0.436	0.170	-0.140	0.157
Q8ZY35: NADH dehydrogenase	DV437845	0.030	-0.036	-0.297	-0.569	-0.110	-0.403	-0.778	0.077	-0.076	-0.705
U36394: Ornithine decarboxylase	ES408263	-0.032	0.112	0.272	2.092	-0.113	0.151	1.113	-0.034	-0.436	0.270
XM_341636: Carboxylesterase	ES108269	0.034	0.432	0.431	1.028	0.029	0.265	0.799	0.246	0.081	1.520
Q7FPX0: Sulfotransferase	DV437802	0.159	-0.026	0.431	0.594	0.241	0.284	0.577	0.094	-0.021	0.010
Q7ZYH0: Sulfotransferase	DV437811	0.185	-0.026	0.382	0.358	0.110	0.250	0.454	0.061	-0.016	0.180
AY572863: Actin	ES408251	-0.022	0.392	-0.167	-1.677	0.524	0.138	0.325	0.259	0.129	0.166
Unknown function											
Q8Z7T2: unknown	DV437814	0.495	0.008	-0.676	0.187	0.557	-0.527	0.403	-0.032	-1.531	0.239
AP006275: unknown	DV437837	-0.205	-0.174	-0.320	-1.438	-0.177	-0.424	-1.217	0.178	-0.074	-1.408
XM_546676: unknown	ES108222	0.010	0.502	0.296	0.683	0.134	0.461	0.276	0.008	0.081	0.674
XM_364913: unknown	ES408299	-0.204	-0.160	-0.285	-1.173	0.038	-0.354	-0.882	-0.134	-0.173	-0.943
Q8I292: unknown	DV437851	-0.226	-0.336	-0.171	-1.130	0.066	-0.636	-0.639	-0.016	0.076	-0.909
Q9VRS7: unknown	DV437855	-0.306	0.133	-0.089	-0.909	0.009	-0.319	-0.626	0.194	0.739	-1.330
AL034583: glycine rich protein	ES108183	0.024	0.096	0.103	1.293	0.031	0.140	0.181	0.150	0.004	0.350
AE003705: unknown	ES408189	0.011	0.107	0.177	1.482	0.016	0.039	1.109	-0.014	-0.460	0.120
Q7Q8R2: unknown	DV437810	-0.025	0.043	0.361	0.490	-0.035	0.352	0.961	-0.175	-0.010	0.897
Q8YA32: unknown	DV437808	0.063	0.692	0.444	1.001	0.007	0.183	1.014	-0.011	-0.225	0.971
Q9VSES: unknown	DV437807	0.060	0.189	0.696	0.584	0.010	0.140	0.489	0.084	0.412	0.194
No homology											
no homology	ES408276	-0.126	-0.342	-0.283	-1.061	0.001	-0.279	-0.889	0.086	0.171	-0.779
no homology	DV437818	0.158	0.039	0.465	1.283	0.302	0.326	0.312	0.210	0.095	0.557
no homology	ES408296	-0.130	-0.471	-0.327	-1.253	0.070	-0.304	-0.957	0.023	0.170	-0.990
no homology	ES408233	-0.005	0.476	0.091	-1.214	0.733	0.144	0.232	0.085	0.197	0.066
no homology	ES408179	-0.739	-0.419	-0.294	-1.079	-0.486	-0.468	-0.563	-0.187	-0.221	-0.722
no homology	ES108296	0.168	0.089	0.444	1.016	0.068	0.045	0.503	0.232	0.033	1.144
no homology	ES408225	-0.147	0.425	-0.137	-0.879	0.805	-0.149	0.362	-0.060	0.063	-0.440
no homology	ES408268	0.090	0.528	-0.014	-0.860	0.541	-0.154	0.571	-0.260	0.176	-0.206
no homology	DV437816	-0.083	-0.166	-0.443	-0.789	0.043	0.047	-0.243	0.142	0.233	-0.670
no homology	ES108223	0.021	0.485	0.146	0.648	0.722	0.147	0.185	0.088	0.076	0.479
no homology	AJ292556	0.037	0.454	0.031	-0.020	0.407	0.100	0.436	-0.168	0.217	-0.403
no homology	ES408221	-0.370	-0.392	-0.162	-0.574	-0.650	-0.323	-0.808	-0.316	-0.150	-0.836
no homology	ES408284	-0.373	-0.657	-0.237	-0.155	-0.153	-0.137	-0.501	0.054	0.142	-0.088
no homology	ES108285	0.236	0.747	0.100	0.925	0.269	0.092	0.180	0.398	0.179	0.409
no homology	ES408269	0.102	0.026	0.106	1.211	-0.110	0.030	0.650	-0.043	-0.100	0.670
no homology	ES408293	-0.377	-0.527	-0.022	1.331	-0.364	0.000	0.149	-0.575	-0.164	0.375
no homology	DV437805	-0.046	0.672	0.437	1.162	-0.031	0.111	1.143	-0.043	-0.250	1.055
no homology	ES108234	0.120	0.136	0.279	1.738	0.189	0.301	0.482	-0.127	0.173	0.026

Six independent microarray hybridizations were carried out for each of exposure concentration.

Candidate differentially expressed genes were determined using the method of Loginov *et al.* (2).

Clones which were differentially expressed in at least 4 of the 6 microarray hybridizations were chosen as candidate differentially expressed genes. Green represents down-regulated differentially expressed genes, and red represents up-regulated differentially expressed genes.

Shown here is the average \log_2 ratio for the six microarray hybridizations.

Table S4: Quantitative Reverse Transcription PCR (q-RT-PCR) confirmation of microarray gene expression ratios

			1/20EC50 Cu	1/10LC50 Cu	NOEC Cu	1/10EC50 Cd	1/10LC50 Cd	NOEC Cd	1/10EC50 Zn	1/10LC50 Zn	NOEC Zn
Monooxygenase	DV437798	μarray	0.10	1.23	2.20	0.01	1.49	1.75	-0.01	0.54	1.52
		q-RT-PCR	1.09	2.32	2.45	0.52	2.87	1.54	0.25	0.00	0.40
GST1	DV437830	μarray	-0.04	0.11	1.06	0.07	0.48	0.73	0.02	0.19	0.01
		q-RT-PCR	1.33	0.49	-0.27	-0.25	1.00	-0.50	0.68	-0.29	-0.40
IMPase	DV437806	μarray	0.14	0.60	0.85	0.06	0.16	0.44	0.01	-0.49	0.57
		q-RT-PCR	0.06	0.49	-0.04	0.23	-0.67	-0.60	0.23	-0.34	-0.20
Cellulase	DV437797	μarray	-0.30	-1.42	-2.47	-0.13	-1.38	-2.06	0.31	-1.11	-2.38
		q-RT-PCR	0.85	-1.31	-2.80	0.19	-1.17	-3.46	0.08	-2.28	-2.83
Slit homolog	DV437805	μarray	-0.05	0.44	1.16	-0.03	0.11	1.14	-0.04	-0.25	1.07
		q-RT-PCR	0.58	3.24	1.26	-0.35	2.44	1.03	0.38	2.32	-0.22
MT (b)	DV437799	μarray	0.20	0.64	1.25	0.12	0.71	0.96	0.00	0.58	0.35
		q-RT-PCR	2.26	2.37	1.53	0.41	1.78	1.22	0.31	1.70	0.24
unknown	ES408276	μarray	-0.13	-0.28	-1.06	0.00	-0.28	-0.69	0.07	0.17	-0.78
		q-RT-PCR	1.63	0.01	-0.92	0.56	-0.28	-1.14	0.49	-0.48	-1.46
Preamylase	ES408209	μarray	0.06	-0.65	-1.18	0.13	-0.29	0.04	0.19	-0.84	-0.70
		q-RT-PCR	1.63	0.19	-1.29	0.39	0.32	-1.32	0.01	-0.33	-1.91
Chitinase	DV437858	μarray	0.10	0.05	-0.14	0.08	0.07	0.93	0.13	-1.12	-0.91
		q-RT-PCR	1.13	-1.47	-2.45	0.45	-0.71	-3.03	1.24	-1.99	-2.64

D. magna were exposed to each exposure concentration for 24-h. Following the exposure, RNA was isolated and reverse transcribed. qPCR was carried out on the cDNA using SYBR Green as described in the methods section. Log₂ ratios from the q-RT-PCR analysis are compared to the averaged log₂ ratios for the microarray experiments. Genes significantly differentially expressed for a given condition are shown in red for upregulated genes or green for downregulated genes.

Table S5: Estimation of the NOTEL

Probability	Concentration (µg/L)	Concentration lower (µg/L)	Concentration upper (µg/L)	Response lower	Response upper
0.0005	0.20	0.07	0.58	1.25	5.00
0.0010	0.58	0.26	1.30	2.93	8.52
0.0015	1.07	0.55	2.09	4.82	11.66

Estimation of the NOTEL is a maximum concentration which gives a response whose 95% CIs are still overlapping with 95% CIs for the estimate of noise (in unexposed versus unexposed controls). For Cu, the estimation for noise was found to be 1.64 [0.000, 3.19] differentially expressed genes at average; therefore, the NOTEL was estimated using the table above to be 0.58 µg/L because it was the highest concentration whose 95% CIs for differentially expressed genes [2.93, 8.52] overlapped with the 95% CI for noise.

Probability - predicted probability to be differentially expressed at a given concentration (response),

Concentration - Concentration on original scale,

Concentration lower - lower 95% confidence limits for concentration,

Concentration upper - upper 95% confidence limits for concentration,

Response lower - lower 95% confidence limits for the response shown as the number of differentially expressed genes,

Response upper - upper 95% confidence limits for the response shown as the number of differentially expressed genes.

References:

1. Rozen, S.; Skaletsky, H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* **2000**, *132*, 365-386.
2. Loguinov, A. V.; Mian, I. S.; Vulpe, C. D. Exploratory differential gene expression analysis in microarray experiments with no or limited replication. *Genome Biol.* **2004**, *5*, R18.

Figure Legends

Figure S1: Exposure and microarray hybridization experimental design. Three replicate exposures (exp) were performed for each metal concentration on separate dates. A zero concentration control (ctrl) was performed alongside each metal exposure, so that each exposure had a complimentary unexposed control for microarray hybridization. Following each 24-hour exposure, *D. magna* were collected and RNA was extracted for microarray hybridizations. Before proceeding to reverse transcription, RNA from both the unexposed and exposed *D. magna* was split into two pools, to provide two dye-swapped technical replicates for each metal exposure. Because three exposures were performed for each metal, and RNA from each exposure was hybridized to two different microarrays, there were six hybridizations for each exposure condition.

Figure S2: Acute and chronic toxicity of metals to *Daphnia magna*. *D. magna* were exposed to varying concentrations of copper (A), cadmium (B), or zinc (C) for 24 hours (acute) or 21 days (chronic). Survival after acute exposure (●) and reproduction after chronic exposure (▲) are shown as percent of the control exposures. * denotes concentrations that cause a significant decrease ($p < 0.05$) in survival or reproduction from the control.

Figure S3: Quantitative Reverse Transcription PCR investigation of metallothionein genes following metal exposure. In each experiment, the expression level of each gene was normalized to 18S rRNA and is shown as the fold induction compared to an unexposed control. Metallothionein gene expression determined by q-RT-PCR is shown for two MT transcripts at different metal concentrations to illustrate the specificity of MT (a) (DV437826) and MT (b) (DV437799) and to confirm the gene expression patterns seen by microarray analysis. Error bars represent the standard deviation across three technical replicates.

Fig. S1

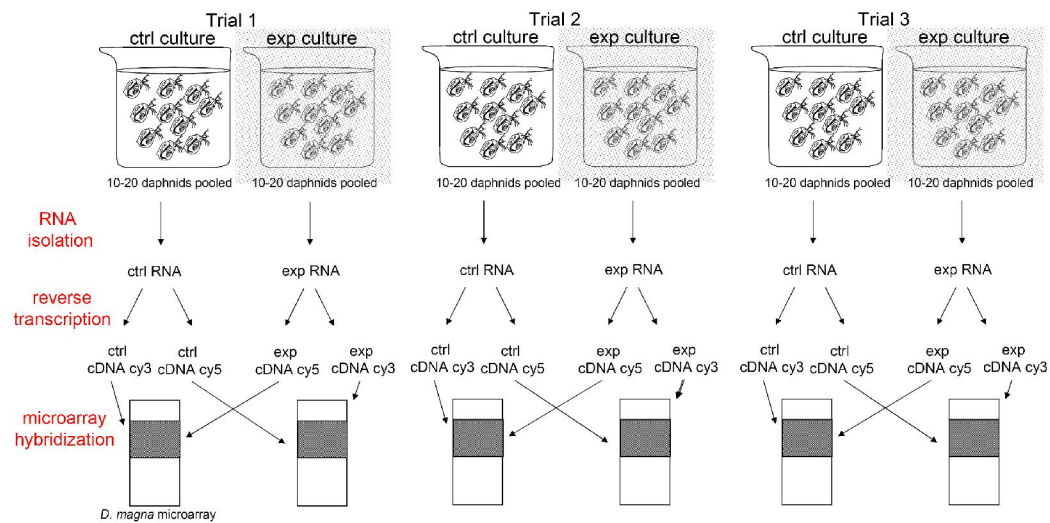


Fig. S2

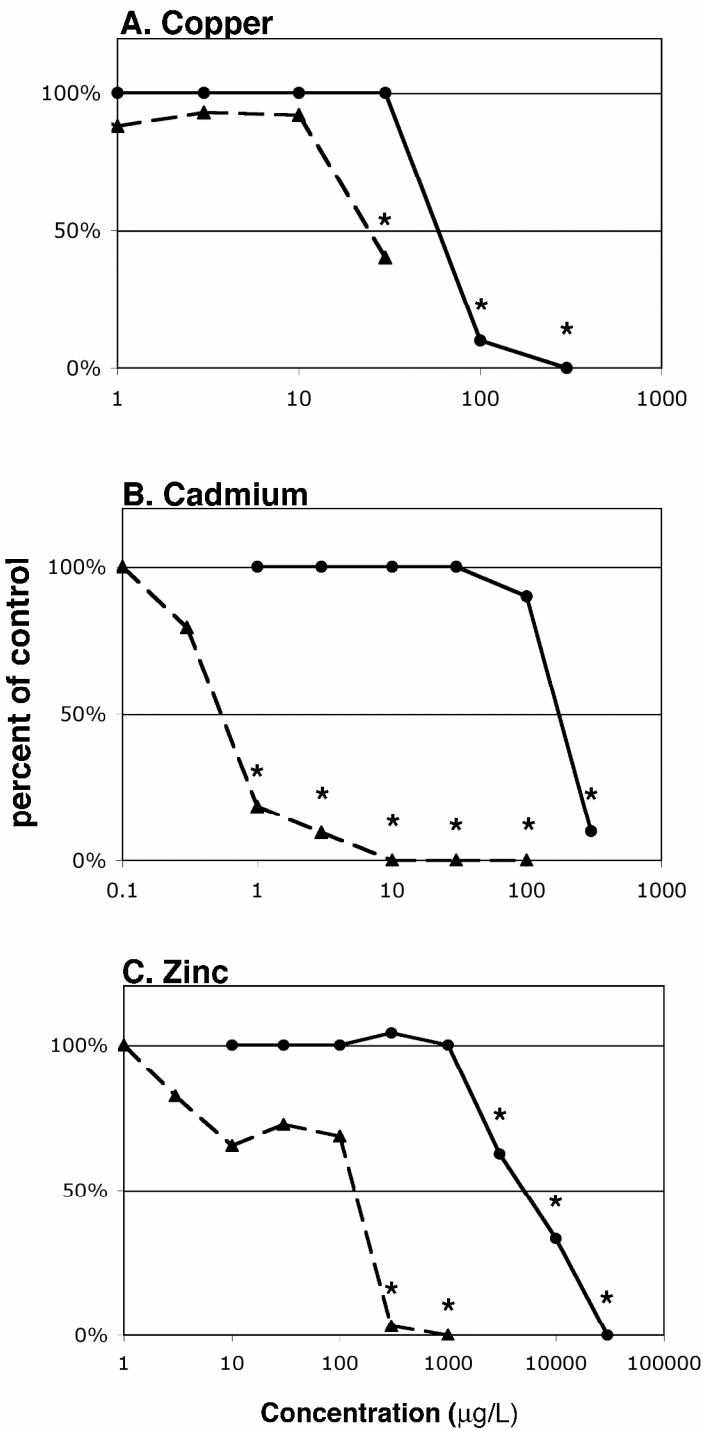


Fig. S3

