Supporting Information

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

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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

Table S1: Description of exposure levels for microarray hybridizations

Exposure levels were chosen based on acute (24-h) and chronic (21-d) toxicity endpoints. These levels were chosen to represent a tolerated concentration ($1/20 \text{ EC}_{50}$ or $1/10 \text{ EC}_{50}$), a sublethal concentration ($1/10 \text{ LC}_{50}$), and a nearly acutely toxic concentration (NOEC).

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

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

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	-0.559
Q26193: Preamylase 1 pre. Q8WPJ2:Endo-β-1,4-mannanase	DV437815 DV437795	-0.169	-0.503	-0.656	-0.979	0.195	-0.333	0.212	0.209	-1.213	-0.802
XM 792007: Hydrolase	ES408267	-0.219 -0.071	-0.581	-0.309	-0.949 -0.903	-0.012 -0.102	-0.253	-0.923	0.378	-0.848	-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
AY081766: Cellobiohydrolase I	ES408211	-0.026	-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	-0.462	0.094	-0.642	-0.143	0.035	-0.587	-0.008
Q8MRH5: Serine protease	DV437844 DV437843	-0.014	0.183	-0.405	-0.462	0.049	-0.638	-0.143	-0.011	-0.587	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	DV437838	-0.200	-0.083	0.123	-1.100	0.028	-0.554	-0.726	0.250	-0.381	-1.072
O46137: Chymotrysin precursor	DV437834	-0.049	0.461	-0.199	-0.854	0.065	-0.548	0.015	0.088	-0.797	0.231
Q8I9P2: Trypsin	DV437840	-0.181	-0.184	-0.250	-1.026	-0.023	-0.469	-0.942	0.130	0.290	-0.825
Q7Q9W3: Serine protease	DV437839 ES408193	0.033	0.170	0.060	-0.428	0.032	-0.484 -0.483	-0.251	0.086	-0.146 -0.212	-0.043
AF486488: Trypsin Q27824: Serine collagenase 4	ES408193 DV437841	-0.211 -0.333	-0.119 -0.203	0.124	-0.493	-0.080	-0.483	-0.821	0.294 0.173	0.397	-1.118
P36178: Chymotrypsin Bll 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	0.306	0.416	-1.221
Q8I9P2 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 AY795054: Endopeptidase	DV437854 ES408379	-0.230 -0.086	0.193 0.046	-0.011 0.340	-0.572 1.414	0.105 0.172	0.058 0.146	-0.286 0.984	0.200 0.012	0.759	-1.075
Metal binding and transport									a.		
Q9U0S3: Ferritin subunit	DV437849	-0.080	-0.187	-0.342	-0.359	0.045	-0.688	-0.625	0.096	-0.330	-0.311
AY297687: Ferritin	DV437852	0.047	-0.325	-0.279	-0.458	-0.009	-0.663	-0.718	0.038	-0.160	-0.215
Q9D8U4: Heavymetal binding	DV437835	-0.085	-0.144	-0.334	-0.768	-0.163	-0.463	-0.196	0.051	-0.385	-0.640
AAC47544: Hemoglobin NM_200198: Heme binding protein	U67067 ES408214	-0.403 -0.040	-0.189 0.333	0.008	-0.551	-0.574 0.049	0.080 0.102	-0.081 0.591	-0.512 0.054	-0.566 -0.235	0.704 0.085
Q9NA01: Ferritin	AJ292556	-0.040	0.355	0.501	0.426	0.049	0.102	0.591	0.054	0.235	-0.039
No homology (MT)	DV437799	0.198	0.813	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	0.864
Exoskeletal proteins	DV/427858	0.108	0.274	0.038	0.020	0.087	0.068	0.128	0.440	1.947	0.403
AAH60393: Cuticle protein Q7PWC2 Chitinase	DV437856 DV437857	0.106	-0.274 -0.116	0.036	-0.020 -0.159	-0.067 0.076	-0.068 0.073	-0.136	-0.449 0.119	-1.247 -1.121	-0.403
AF026491: Chitinase	DV437858	0.096	-0.122	0.054	-0.138	0.079	0.073		0.126	-1.123	-0.913
Q7PRG6: Chitin binding	DV437800	-0.147	-0.021	-0.690	-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	0.496	-0.109	0.093	0.409	-0.347
P91731: Chitinase	DV437809	0.131	0.228	0.411	-0.253	0.059	-0.306	-0.062	0.176	-0.393	0.000
XM_318997: Cuticle protein	ES408180	0.231	-0.092	-0.176	-0.854	0.529	-0.055	0.257	0.501	-0.153	-0.036
EF203462: Cuticle protein XM_318987: Cuticle protein	ES408177 ES408178	-0.202 -0.029	0.555	0.071	-1.191	0.814	0.106	0.294	0.411	0.047	0.131
XM_001119869: Cuticle	ES408185	-0.051	0.472	-0.036	-0.944	0.893	0.158	0.363	0.341	0.132	0.158
AE003563: Cuticle protein	ES408176	-0.146	0.499	-0.080	-1.749	0.664	0.035	0.029	0.297	0.192	-0.033
AY432732: Cuticle protein	ES408184	-0.242	0.911	-0.135	-1.668	1.517	0.030	0.124	0.396	0.171	0.419
Reproductive function and deve CR688752: Placental protein	elopment ES408215	-0.191	-0.618	-0.406	-0.313	-0.067	-0.413	-1.041	0.170	-0.591	0.062
BAD05137: Vitellogenin SOD	AB114859	-0.294	-0.523	-0.406	-0.836	-0.067	-0.413	-1.041	0.025	-0.591	-0.938
U56966: Vitellogenin	ES408219	-0.455	-0.610	-0.190	-1.527	-0.690	-0.452	-1.007	-0.158	-0.152	-0.799
AB252738: Vitellogenin-SOD2	ES408217	-0.869	-0.632	-0.218	-0.700	-0.711	-0.277	-0.912	-0.384	-0.255	-0.853
AB114859: VTG-SOD	AB114860	-0.318	-0.388	-0.232	-0.880	-0.214	-0.314	-0.788	-0.073	-0.118	-0.850
Q94440: Posterior end mark	DV437824	0.103	-0.252	-0.524	-0.520	0.456	-0.582	-0.668	-0.034	-0.401	-0.785
Q9VEY6: Hormone reg. peptidase Q7Z407: CUB and sushi	DV437846 DV437847	0.045	0.334 0.346	0.282	-0.082 -0.412	0.044 -0.020	-0.640 -0.335	-0.353 -0.293	-0.017 0.035	-0.332 0.021	0.187 -0.132
Monooxygenases											
NM_000016: Acyl coA dehydrogenase	ES408245	0.070	0.988	-0.049	1.884	0.848	0.053	0.367	0.325	-0.084	0.356
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 Q8BUZ7: Monooxygenase X	DV437801 DV437798	0.298	-0.074 1.023	1.107 . 1.251	1.159 2.271	0.123	0.277	0.000	0.198	-0.774 0.548	-0.740 1.567
Immune Function											
Q7QCT4:8-1,3-glucan binding Q8IUN9: Macrophage lectin	DV437823 DV437813	-0.251	-0.809	-0.756	-1.839 -0.613	0.019	-0.299	-0.849 -0.394	0.274	-0.287	-1.531 -0.833
Oxidative Stress Response	54407010		-0.050	0.440	-0.010	-0.011	0.200		-0.110	0.101	-0.000
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	DV437829 DV437830	-0.038	0.051	0.176	1.060	0.074	0.482	0.425	0.040	0.347	0.031
Q9NAW7: Glutathione S-transferase	DV437833	-0.017	0.391	0.306	0.118	0.067	0.689	0.541	0.033	0.380	0.034
Cell signaling	DI (1070.40	0.400	0.407		1.001	0.400	0.747	0.07/	0.400		1710
Q9PT14: Precerebellin-like Q9VAG9: Inositol monophosophatase	DV437848	-0.122	-0.127	-0.486	-1.291	-0.106	-0.747	-0.674	0.192	-0.623	-1.748
P51150: Ras-related, Rab-7	DV437806 DV437832	0.140 0.072	0.264 0.529	0.604	0.848 1.388	0.060 -0.041	0.156	0.441	0.014 0.072	-0.487 0.261	0.570
XM 647306: Cell division kinase	ES408226	-0.112	0.329	0.335	1.238	0.104	0.435		-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

Lipde Adverts/05 System -0.13 -0.140 -0.320 -1.122 -0.031 -0.460 -0.032 -0.133 -0.160 -0.226 -0.22	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
Oddess, Hear fairy acd binding DV33917 -0 461 -0 411 -0 522 -0 734 -0 073 -0 056 -0 173 -0 464 -0 464 AV33936: E-tark acd binding E-546521 -0 378 0 423 0 423 0 444 0 374 0 484 0 464	Lipid metabolism and transport											
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AA13948- Faity and bending UV437814 V272024 Faity and bending UV437814 AV22024 Faity and bending UV437818 AV22024 Faity and bending UV437814 AV22024 Faity and bending UV437814 AV2204 Faity and bending UV437814	Q91896: Heart fatty acid binding					-0.734		-0.353	-0.849		-0.286	-0.866
Other functions Opd24 0.578 0.684 0.644					-0.192							
AA03982: Instremenmoluchia DV437831 AA0447: Clammine synthesse-likk E540620 AV32247: Clammine synthesse-likk E540620 AV3247: Clammine synthesse-likk E540620 AV3247: Clammine synthesse-likk E540620 AV3247: Clammine synthesse-likk E540620 AV3247: Clammine synthesse-likk E540620 AV3240: Clammine synthesse-likk E540620 AV3440: Clammine synthesse-likk E540620 AV34404: Likk AV444 AV32452: Likk AV444 AV32452: Likk AV444 AV32452: Lik	AY279214: Fatty acid binding	ES408221	-0.370	-0.429	-0.218	-0.493	-0.484	-0.374	-0.818	-0.406	-0.262	-0.786
AA03982: Instrinon - moluchia DV437831 AA0447: Clammine synthess-likk E540520 AV32247: Clammine synthess-likk E540529 AV32247: Clammine synthess-likk E540529 AV32247: Clammine synthess-likk E540529 AV32247: Clammine synthess-likk E540529 AV32247: Clammine synthess-likk E540529 AV32251: NAOH delykiopenses DV43764. AV3251: NAOH delykiopenses DV43764. AV32524. AV3251: NAOH delykiopenses DV43764. AV32524. AV3255. AV3254. AV3254. AV3255. AV3254. AV3254. AV3255. AV3254. AV3255. AV3254. AV3255. AV3254. AV3255. AV3254. AV3255. AV3254. AV3255. AV3254. AV3255. AV3254. AV3255. A												
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AV32247: Clutamine subclobe Control E5402250 0-0119 0.883 -0.044 0-1716 0.027 0.031 0.272 0.011 0.384 NM 137262: Control E5402259 0.076 0.014 -1422 0.002 0.434 0.778 0.029 0.014 0.411 0.272 0.001 0.384 VM 137262: Control E540259 0.077 0.027 0.439 0.776 0.0776	AAQ83892: Interferon y-inducible	DV437831	-0.924	0.378	0.358	0.534	0.114		0.836	0.059	0.079	
M1 37528: Guanne nucleotede exchar ES-4082.33 -0.094 0.823 0.014 -1.442 0.008 0.141 0.471 0.203 0.009 0.015 0.078 0.009 0.176 0.077 0.027 0.028 VM1 59582. Acpatita aminformatienes ES-6082.43 0.072 0.521 0.010 -0.463 0.177 -0.077 -0.076 -0.766 Q2735 IX-D01 Height delyndugenase ES-4082.43 0.032 0.112 0.227 0.028 0.013 0.181 1.113 -0.034 -0.034 -0.034 -0.043 -0.024 0.028 0.027 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.025 0.016 -0.016 0.160 1.160 0.077 0.074 -0.022 0.016 -0.016 0.016 -0.021 0.016 -0.022 0.025 0.016 -0.021 0.025 0.016 -0.021 0.024 0.025 0.016 -0.021 0.025 0.016 -0.021 0.025 0.027 0.423 -0.022 0.025 0.016 -0.021 0.025	AJ304447: Serine protease inhibitor	ES408189	-0.063	0.116	0.148	1.506	0.130	0.046	0.927	-0.010	-0.333	0.284
NM 106989, Appolate animotransferase E5408259 0.176 0.127 0.027 0.048 0.027 0.048 0.038 0.000 0.176 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.028 0.027 0.044 0.001 0.016 0.026 0.010 0.027 0.044 0.001 0.016 0.026 0.027 0.028 0.033 0.011 0.226 0.014 0.006 0.017 0.027 0.043 0.018 0.016 0.016 0.016 0.0				0.885								
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Cd2Y35 NADH dehrundrugenase D V33784 O 033 -0.037 -0.038 -0.077 -0.077 -0.077 -0.078 -0.043 -0.077 -0.078 -0.043 -0.0270 -0.043 -0.0270 -0.043 -0.0270 -0.043 -0.0270 -0.043 -0.0270 -0.043 -0.0270 -0.043 -0.0270 -0.043 -0.0270 -0.044 -0.028 -0.028 -0.024 -0.021 -0.023 -0.043 -0.021 -0.022 -1.031 -0.023 -1.031 -0.023 -1.031 -0.023 -1.031 -0.023 -1.031 -0.023 -1.031 -0.033 -0.016 -0.016 </td <td></td>												
U38394: Onthine decarboylase E54082.63 0.012 0.272 2.0024 0.013 0.151 1.113 -0.034 -0.034 0.435 0.270 VXI 341636: Corboylasterase DV437002 0.156 0.020 0.244 0.264 0.677 0.034 0.084 0.081 0.010 0727H0.3.0101srsferase DV437102 0.156 -0.022 0.352 0.110 0.220 0.444 0.661 0.084 -0.010 0.010 0.025 0.128 0.325 0.259 0.128 0.167 0.032 0.457 0.032 0.457 0.032 0.457 0.032 0.457 0.032 0.167 0.032 0.451 0.074 1.418 0.405 Q267712: unknown DV437814 0.445 0.0274 0.418 0.451 0.0274 0.043 0.074 1.418 0.473 0.074 1.418 0.417 0.033 0.074 0.148 0.451 0.0774 0.403 0.074 1.4183 0.068 0.1714 0.425 0.171 </td <td></td> <td></td> <td></td> <td>0.001</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>				0.001								
XML 341536: Carboxylatomaa EE/09269 0.034 0.431 1.028 0.029 0.255 0.799 0.246 0.081 1.560 Q77KU0. Sulformsforase DV437811 0.165 -0.028 0.421 0.224 0.257 0.644 0.077 0.644 0.077 0.644 0.077 0.644 0.071 0.644 0.061 -0.016 0.100 Q77KU0. Sulformsforase DV437811 0.022 0.392 0.167 1.677 0.624 0.277 0.644 0.077 0.032 -1.153 0.239 Unknown function DV437814 0.495 0.008 -1.677 0.557 -0.627 0.403 -0.032 -1.531 0.239 VML 54675: kinknown E5408222 0.100 0.502 0.289 -0.174 -0.232 -0.177 -0.424 -0.628 -0.173 -0.032 -1.631 0.239 VML 54675: kinknown E5408229 -0.204 -0.160 -0.255 -1.173 0.036 -0.174 -0.173 -0.174						-0.569						
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Q2772: unknown DV437814 0.495 0.002 -1.631 0.239 AP006775: unknown DV437837 -0.205 -0.174 -0.320 -1.438 -0.177 -0.427 0.403 0.032 -1.431 0.239 XM_546575: unknown E5408222 -0.010 0.502 -0.296 0.683 0.134 -0.461 0.276 0.008 0.081 0.974 XM_546575: unknown DV437851 -0.225 -1.173 0.038 -0.054 -0.002 -0.134 -0.173 -0.943 G2027: unknown DV437851 -0.225 -0.338 -0.0171 -1.130 0.066 -0.635 -0.014 0.172 -1.133 0.069 -0.319 -0.626 0.194 0.172 -1.330 -0.064 -0.638 -0.014 -0.040 0.350 CSVTRS: unknown DV437810 -0.025 0.043 1.055 0.031 0.140 0.181 0.160 0.024 0.0225 0.981 -0.014 -0.040 0.350 CGV3E5: unknown	Unknown function											
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XM_54675: unknown E5408222 0.010 0.502 0.285 0.134 0.441 0.276 0.008 0.081 0.074 XM_304913 unknown E5408299 -0.226 -0.136 -0.254 -0.082 -0.114 -0.173 -0.043 QBU92: unknown DV437851 -0.226 -0.338 -0.054 -0.0626 0.076 -0.069 QSVR57: unknown E5408183 0.024 0.030 1.0140 0.181 0.150 0.044 0.250 -1.330 AL034563: unknown E5408199 0.011 0.107 0.171 1.402 0.016 0.033 1.109 -0.014 -0.400 0.120 QFVR32: unknown DV437808 0.083 0.692 0.444 1.001 0.007 0.183 1.014 -0.014 -0.225 0.571 QFVR32: unknown DV437808 0.693 0.692 0.444 1.001 0.007 0.183 1.041 -0.011 -0.225												
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AL02463: glyaine rich protein E5408183 0.024 0.096 0.103 1.903 0.031 0.140 0.131 0.150 0.004 0.350 AE003705: unknown E5408189 0.011 0.107 0.177 1.402 0.016 0.033 1.000 -0.014 -0.406 0.120 GQVSE5: unknown DV437810 0.063 0.692 0.444 1.001 0.007 0.133 1.014 -0.014 -0.225 0.971 GVSE5: unknown DV437808 0.063 0.692 0.444 1.001 0.007 0.133 1.014 -0.014 -0.225 0.971 GVSE5: unknown DV437808 0.0663 0.482 -0.283 -1.061 0.010 0.140 0.489 0.412 0.171 -0.279 -0.689 0.066 0.171 -0.799 no hamology 0.160 0.033 -0.271 -0.056 0.657 No hamology E5408233 -0.005 0.476 0.091 -1.213 0.07	Q9VRS7: unknown											
AEco3705: unknown E5408189 0.011 0.107 0.177 1.402 0.016 0.033 1.100 -0.014 -0.400 0.120 Q7Q8R2: unknown DV437810 -0.025 0.043 0.301 0.490 -0.035 0.352 0.981 -0.175 -0.010 0.897 Q8Y32: unknown DV437807 0.060 0.432 0.444 1.001 0.007 0.183 1.014 0.011 -0.225 0.971 Q8VSE5: unknown DV437807 0.060 0.489 0.602 0.584 0.010 0.140 0.489 0.064 0.412 0.194 No homology DV437816 0.029 0.425 -1.261 0.001 -0.279 -0.889 0.0668 0.171 -0.779 no homology DV437818 0.013 -0.471 -0.327 -1.253 0.070 -0.344 0.957 0.023 0.176 -0.956 No homology E5408236 -0.479 -0.468 -0.468 -0.463 -0.187 -0.221	AL034563: glycine rich protein	ES408183	-0.024	0.096	0.103	1.293	-0.031	0.140	0.181	-0.150	0.004	0.350
Carva32: unknown DV437808 0.083 0.692 0.444 1.001 0.007 0.183 1.014 -0.011 -0.225 0.971 CgVS25: unknown DV437807 0.060 0.189 0.066 0.584 0.010 0.140 0.489 0.084 0.412 0.144 No homology ES4082/6 0.158 0.039 0.445 1.283 0.302 0.328 0.312 0.210 0.095 0.557 No homology DV437818 0.158 0.039 0.445 1.283 0.302 0.328 0.312 0.210 0.095 0.557 No homology ES408299 -0.138 -0.471 -0.277 -1.223 0.070 -0.344 -0.957 0.023 0.170 -0.666 No homology ES408179 -0.799 -0.488 -0.488 -0.963 -0.187 -0.221 -0.722 0.722 0.722 0.722 0.722 -0.722 0.722 0.722 0.722 0.722 0.722 0.722 0.722 <td>AE003765: unknown</td> <td>ES408189</td> <td>0.011</td> <td>0.107</td> <td>0.177</td> <td>1.482</td> <td>0.016</td> <td>0.039</td> <td>1.169</td> <td>-0.014</td> <td>-0.460</td> <td>0.120</td>	AE003765: unknown	ES408189	0.011	0.107	0.177	1.482	0.016	0.039	1.169	-0.014	-0.460	0.120
CGVSE5: unknown DV437807 0.080 0.188 0.055 0.684 0.010 0.140 0.489 0.084 0.412 0.194 No homology DV437807 0.080 0.188 0.055 0.684 0.010 0.140 0.489 0.084 0.412 0.194 No homology DV437818 0.158 0.0342 -0.2283 -1.061 0.001 -0.279 -0.689 0.096 0.171 -0.779 no homology DV437818 0.158 0.0321 1.283 0.002 0.328 0.0210 0.095 0.657 No homology E5408233 -0.005 0.471 -0.221 -1.079 -0.468 -0.563 -0.187 0.021 0.0720 -0.722 No homology E5408233 -0.005 -0.419 -0.279 -0.468 0.563 0.187 -0.221 -0.722 No homology E5408233 -0.0169 0.444 1.016 0.966 0.448 0.563 0.222 0.003 1.442	Q7Q8R2: unknown	DV437810	-0.025	0.043	0.361	0.490	-0.035	0.352	0.981	-0.175	-0.010	0.897
No homology no homology ES408276 ES408296 -0.125 -0.342 -0.283 -1.061 0.001 -0.279 -0.689 0.096 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 -0.138 -0.471 -0.327 -1.253 0.070 -0.304 -0.957 0.023 0.170 0.990 0.665 0.990 0.665 0.990 0.055 0.023 0.170 0.990 0.065 0.197 0.065 0.917 -0.722 0.070 -0.304 -0.957 0.023 0.170 0.990 0.065 0.018 -0.197 -0.721 -0.722 1.072 1.073 0.144 0.252 0.018 0.049 0.323 1144 0.322 0.033 1144 0.321 1.072 1.072 1.072 1.072 1.072 1.072 1.072 1.072 1.072 1.072 1.072 1.072	Q8YA32: unknown	DV437808	0.063	0.692	0.444	1.001	0.007	0.183	1.014	-0.011	-0.225	0.971
no homology ES4082/76 -0.126 -0.342 -0.283 -1.061 0.001 -0.279 -0.689 0.068 0.171 -0.779 No homology DV437818 0.158 0.036 0.4655 1.283 0.002 0.326 0.312 0.210 0.069 0.690 No homology ES408233 -0.050 0.476 0.091 -1.214 0.733 0.144 0.232 0.065 0.176 -0.990 No homology ES408233 -0.050 0.476 0.091 -1.214 0.733 0.144 0.232 0.065 0.187 -0.271 0.072 No homology ES408233 -0.059 -0.449 0.079 -0.468 0.663 0.187 0.221 -0.722 No homology ES408266 0.188 0.099 0.444 1.016 0.966 0.045 0.633 0.222 0.033 1.444 no homology ES408286 0.990 0.731 -0.014 0.664 0.571 -0.260 0.477 <td>Q9VSE5: unknown</td> <td>DV437807</td> <td>0.060</td> <td>0.189</td> <td>0.595</td> <td>0.584</td> <td>0.010</td> <td>0.140</td> <td>0.489</td> <td>0.084</td> <td>0.412</td> <td>0.194</td>	Q9VSE5: unknown	DV437807	0.060	0.189	0.595	0.584	0.010	0.140	0.489	0.084	0.412	0.194
no homology ES4082/76 -0.126 -0.342 -0.232 -1.061 0.001 -0.279 -0.689 0.066 0.171 -0.770 No homology DV437818 0.158 0.030 0.455 1.283 0.002 0.326 0.312 0.210 0.065 -0.700 No homology ES408296 -0.138 -0.476 0.091 -1.214 0.733 0.144 0.222 0.085 0.176 -0.090 No homology ES408233 -0.050 0.476 0.091 -1.214 0.733 0.144 0.222 0.085 0.187 0.0722 No homology ES408233 -0.059 0.444 1.079 -0.466 0.048 0.663 0.187 0.0722 0.033 1.444 no homology ES408286 0.090 0.0414 1.046 0.066 0.045 0.503 0.222 0.033 1.444 no homology ES408286 0.090 0.031 -0.147 0.243 0.047 -0.243 0.142 </td <td></td>												
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No homology ES408296 -0.130 -0.471 -0.327 -1.233 0.070 -0.344 -0.957 0.023 0.170 0.990 No homology ES408233 -0.005 0.476 0.091 -1.214 0.733 0.144 0.222 0.085 0.197 0.065 No homology ES408233 -0.016 0.476 0.091 -1.214 0.733 0.144 0.222 0.085 0.197 0.065 No homology ES408296 0.168 0.069 0.444 1.016 0.066 0.045 0.633 0.222 0.003 1.440 no homology ES408286 0.090 0.422 -0.017 0.079 0.005 0.143 0.022 0.003 1.440 no homology ES408288 0.090 0.422 -0.014 0.066 0.441 0.511 -1.220 0.175 -0.206 no homology ES408288 0.090 0.422 -0.033 -0.046 0.541 -0.147 0.142 0.233												
No homology E5408233 -0.005 0.476 0.091 -1.214 0.733 0.144 0.232 0.085 0.197 0.065 no homology E5408237 -0.2780 -0.2194 -1.079 -0.468 -0.468 -0.663 -0.187 -0.222 -0.722 No homology E5408275 -0.147 0.455 -0.137 -0.079 0.006 0.044 0.503 0.222 0.033 1144 no homology E5408225 -0.147 0.455 -0.137 -0.079 0.005 -0.149 0.302 -0.000 0.033 -0.440 no homology E5408288 0.090 -0.044 -0.860 0.541 -0.154 0.571 -2.280 0.162 -0.243 0.142 -0.280 0.162 -0.679 0.043 0.047 -0.243 0.142 -0.231 -0.469 -0.443 -0.789 0.043 0.047 -0.145 0.088 0.727 -0.469 -0.479 -0.469 0.422 0.147 0.185 0.												
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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 log₂ ratio for the six microarray hybridizations. Table S4: Quantitative Reverse Transcription PCR (q-RT-PCR) confirmation of microarray gene expression ratios

			1/20EC50	1/10LC50	NOEC	1/10EC50	1/10LC50	NOEC	1/10EC50	1/10LC50	NOEC
			Cu	Cu	Cu	Cd	Cd	Cd	Zn	Zn	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 μ g/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.

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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 (\bullet) and reproduction after chronic exposure (\blacktriangle) 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







