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RARE METALS INC.

Avalon Rare Metals Inc.

RESPONSE TO THE APRIL 16, 2012 CLARIFICATIONS LETTER FROM MACKENZIE VALLEY ENVIRONMENTAL IMPACT REVIEW BOARD FOR THE THOR LAKE RARE EARTH ELEMENT PROJECT DEVELOPER'S ASSESSMENT REPORT

Submitted To:
MACKENZIE VALLEY ENVIRONMENTAL IMPACT REVIEW BOARD

May 10, 2012

Avalon Rare Metals Inc. (Avalon) is pleased to provide the following responses to the Mackenzie Valley Environmental Impact Review Board (MVEIRB) letter dated April 16, 2012.

Avalon's responses are found after each information clarification request. For consistency, Avalon has kept the same request and response numbers as previously submitted in the April 2, 2012 submission, except for the new #4 (Footnote), which is a response to the MVEIRB request that appeared as a footnote.

IR Clarification:	**#1**
Source:	MVEIRB
To:	Avalon Rare Metals Inc.

Preamble

Items #1 and #3 from the 22-March-12 letter also remain outstanding.

MVEIRB IR Clarification Request #1

Please submit results of both sublethal and chronic toxicity tests (as well as the associated acute toxicity test) on a sample of simulated effluent from the March 2012 pilot plant testing Avalon refers to in both Avalon Response #3.1 (in response to AANDC's IR# 3.1) and Avalon Response #23 (Environment Canada's IR#23).

Avalon IR Clarification Response #1

Avalon is pleased to provide its response to the above IR Clarification request and is providing the results of the acute and sub-lethal/chronic toxicity testing. Copies of all recent laboratory test results discussed in this response are provided in Attachment 1.

As stated in the submission of March 28, the flotation pilot plant testing objectives included producing sufficient concentrate for hydrometallurgical testing. Not unexpectedly, reagent consumption used was well above the expected operational consumption to ensure sufficient recovery, and resulted in elevated concentrations of some reagents in the final effluent. Avalon further stated that further flotation testing is ongoing to optimize (reduce) the reagent usage. Preliminary results have identified significant reduction and potential elimination of some reagents.

Using the pilot plant water, Avalon simulated the holding period for the effluent in the tailings management area consistent with the time-frame identified in the DAR. It then subjected the effluent to standard flotation plant treatment methodologies to remove these reagents to the extent practicable by proven technologies. These technologies are being incorporated into the project design, also as per commitments made by Avalon in the DAR.

Avalon reiterates that it continues to be successful in its ongoing work to refine the floatation process with either reduction or elimination of reagent use. Avalon further clarifies that if the ongoing optimization testing results in reagent use that eliminates the need for the water treatment system identified above, it can easily be removed from the design.

Testing Completed

Given this regulatory regime and the MVEIRB request, Avalon tested the following three waters for both acute and sub-lethal/chronic toxicity:

1. Treated effluent.
2. Treated effluent blended with Drizzle Lake water - The amount of Drizzle Lake water was conservatively set at approximately 90% of the expected Lake:treated effluent ratio as per the hydrology model presented in the DAR (i.e., less Drizzle Lake water than is expected to be available for mixing). Drizzle Lake has been discussed as the location within which CCME guidelines parameter concentrations will be targeted.
3. Drizzle Lake water - It has been reported that natural baseline water quality in Drizzle Lake does not consistently meet CCME guidelines.

All testing was done by AquaTox Testing and Consulting Inc., an independent Canadian laboratory certified for this testing.

The acute toxicity testing was conducted on Rainbow trout and *Daphnia magna* as per the Environment Canada and MMER protocols. An LC_{50} is the concentration of effluent in which 50% of the organisms do not survive over a prescribed time period. Avalon targets 100% survival.

In addition, the following sub-lethal/chronic toxicity tests were completed and assessed using the industry standard IC_{25} (Inhibition Concentration) test, which is the concentration at which there is a 25% impairment of the parameter (e.g. growth rate or reproduction) being tested.

1. Test of larval growth and survival using Fathead minnow. (This also includes an acute toxicity component) The highest score available is an IC_{25}>100%, which indicates that full strength effluent does not result in more than a 25% reduction of growth or reproduction.
2. Test of Reproduction and Survival using the invertebrate *Ceriodaphnia dubia* (This also includes an acute toxicity component) The highest score available is an IC_{25}>100%.
3. Growth inhibition using freshwater algae *Pseudokirchneiriella subcapitata*. The highest score available in this test is an IC_{25} >90.91%.
4. Growth inhibition using freshwater macrophyte *Lemna minor* (duck weed). The highest score available is >97%.

Acute Toxicity Testing Results

Acute toxicity testing resulted in 100% survival of *Daphnia magna*, rainbow trout, fathead minnow, and *Ceodaphnia dubia* (an invertebrate) in all waters tested. Rainbow Trout are among the most sensitive fish species, especially when compared with the fish species in Murky and Thor Lake, the two lakes immediately downstream of Drizzle Lake. These results are attached.

Acute toxicity testing that took place as part of sublethal/chronic toxicity testing also resulted in 100% survival of fathead minnows and *Cerodaphnia dubia*.

In summary, all acute toxicity testing, including 100% effluent, resulted in 100% survival of test organisms.

Sub-Lethal/Chronic Toxicity Testing

While there are no legal criteria for the sub-lethal/chronic toxicity tests, Avalon's assessments and reporting will be based on results from the IC_{25} tests. The following results were obtained from testing that has been completed to date.

The IC_{25} for the fathead minnow was >100% in the final effluent, effluent mixed with Drizzle Lake water, and Drizzle Lake. Results in all three tests were very similar.

With respect to the sub-lethal/chronic reproduction test for the invertebrate *Cerodaphnia dubia*, some inhibition of reproduction rate was observed in the treated effluent (IC_{25}=63.5%), less inhibition was noted for the effluent/Drizzle Lake blend (IC25=75%), and about a 15% inhibition was reported for the Drizzle Lake water only, though this equates to an IC_{25} of >100%. It is noted that there were no mortalities in the final effluent test, while there were some in the blended waters and Drizzle Lake water, but interestingly, only in diluted samples and not in the 100% concentration sample. It is also noted that all three waters behaved in a similar manner, with the impairment only in the sample of undiluted solutions.

The *Pseudokirchneiriella subcapitata* (algae) sub-lethal/chronic growth rate tests suggest that growth stimulation may have occurred as opposed to an impairment of the growth rate. All waters tested had IC25 values greater than 90.91% .

Sublethal/chronic toxicity tests involving the aquatic macrophyte *Lemna minor* have resulted in IC_{25} values greater than 97% for all waters tested.

Water Quality Analysis Results

Water quality analysis results were consistently within federal regulated limits established by the Metal Mining Effluent Regulations (MMER). In addition, results for the treated effluent were also within Canadian Council of Ministers of the Environment (CCME) guidelines for the protection of freshwater aquatic life in the receiving environment for all measured parameters, with the exception of fluorine. This compares with levels of fluorine, suspended solids, cadmium, iron, zinc and ammonia, which exceeded CCME guidelines in background samples of Drizzle Lake water. . Fluorine is significantly higher in the natural lake water, such that the effluent marginally improves the lake water quality respecting this element, suggesting that the host rock within the drainage may be a source of the fluorine.

Summary and Conclusions

The reported analytical results do not account for natural amelioration occurring as a result of physical, biological, and chemical processes in the natural environment. Such processes have the potential to further reduce the potential for acute and sub-lethal effects on aquatic organisms.

All acute toxicity tests achieved 100% survival of the test species. Three of the four sublethal/chronic tests showed no observable effect based on IC_{25} results. The reproduction test showed only minor differences in potential impact between the effluent blended with Drizzle Lake and the Drizzle Lake water and at only the highest concentration tested. Given the available blending in the receiving waters, natural amelioration anticipated upstream of the Drizzle Lake discharge, the natural exceedences of CCME guidelines in Drizzle Lake, and the sensitivity and nature of these tests and test results, this information strongly indicates that there will be no impact on downstream water bodies.

IR Clarification:	**#2**
Source:	MVEIRB
To:	Avalon Rare Metals Inc.

Preamble

On the subject of water quality objectives: the Review Board notes that environmental assessment is the appropriate forum for establishing water quality objectives.

The Review Board recognizes that Avalon – through its 2-April-12 letter – has proposed site-specific water quality objectives for those parameters that CCME (Canadian Council of Ministers of the Environment) guidelines cover including the location where Avalon proposes to meet the objectives: Drizzle Lake outflow. With regard to Avalon's reference to CCME guidelines as being site-specific water quality objectives (SSWQOs) for the Thor Lake Project mine-site – items #2a and #2b in the Review Board's 22-March-12 letter – the Review Board notes that CCME guidelines do not fully encompass the effluent Avalon would likely discharge downstream. In other words: for Avalon's Thor Lake Project, CCME guidelines represent an incomplete/ deficient suite of site-specific water quality objectives. For example, CCME guidelines are silent for lanthanum and cerium: these two parameters are likely to be present in the effluent Avalon proposes to discharge into the downstream environment1. As such, items #2a and #2b from the 22-March-12 letter remain outstanding (including for corresponding rationale) for all elements, parameters, and substances that are likely present in the effluent Avalon proposes to discharge downstream but not covered through CCME guidelines.

MVEIRB IR Clarification Request #2

Please adequately address Aboriginal Affairs and Northern Development Canada's (AANDC) Information Request #1 by submitting:

a. appropriate site-specific water quality objectives specific to the effluent likely to be discharged from the Thor Lake Rare Earth Element Project; and
b. specific location(s) where Avalon proposes to meet the site-specific water quality objectives.

Avalon IR Clarification Response #2(a/b)

Avalon is pleased to advise that following receipt of the Board's April 16, 2012 letter on the three remaining pre-requisite items needed for the conduct of the technical sessions, EBA on behalf of Avalon proceeded to make contact with AANDC and Environment Canada (EC) to initiate discussions leading to the eventual development of site-specific water quality objectives (SSWQOs) for the receiving waters located downstream of the proposed Nechalacho Mine Tailings Management Facility.

Based on these contacts with AANDC and EC, an initial SSWQOs teleconference meeting was convened by AANDC on April 20th, 2012 to initiate discussions on this subject. To assist with the initial discussions, EBA provided a brief Technical Memo to AANDC and EC on April 18th, 2012, prior to the meeting, which summarized the existing background receiving water quality conditions of the Nechalacho Mine area. (Attachment 2 to this response).

The teleconference meeting was held on April 20th, 2012, and the notes of that meeting (dated May 1st, 2012) are provided as Attachment 3 to this response. As indicated in the meeting notes, the discussion concluded with a general consensus that the meeting was productive, that the CCME criteria could form an initial starting point for further discussions for the establishment of SSWQOs for this project, and that future meeting(s) would be useful to resolve issues related to the setting of SSWQOs for other parameters for which CCME criteria do not yet exist.

In particular, it was noted that the next round of baseline surface and groundwater sampling was being undertaken currently (April 2012) and that analysis of rare earth metals (REEs) would be undertaken to assist in establishing baseline conditions for these parameters. These results, which are also provided with this response document to the MVEIRB, will be used in subsequent meetings with AANDC and EC to help with the establishment of SSWQOs for these parameters.

In addition, to further facilitate dialogue on this matter, EBA provided a copy of a recently released report prepared by Wilfrid Laurier University entitled *Review of Aquatic Effects of Lanthanides and Other Uncommon Elements* to AANDC and EC. A copy of this report is provided as Attachment 4 to this response.

IR Clarification: #3
Source: MVEIRB
To: Avalon Rare Metals Inc.

Preamble

Items #1 and #3 from the 22-March-12 letter also remain outstanding. For clarification, Item #3 refers to identification of all water quality parameters present in the Item #1 test-effluents.

MVEIRB IR Clarification Request #3

Please submit the full suite of water quality test results associated with the March 2012 pilot plant testing Avalon refers to in both Avalon Response #3.1 (in response to AANDC's IR# 3.1) and Avalon Response #23 (Environment Canada's IR#23).

Avalon IR Clarification Response #3

As requested, Table 1 provided with this response presents the full suite of water quality testing that was conducted on the three solutions subjected to acute and sublethal testing as referred to in Avalon's response to IR Clarification Response 1:

- PP 22 BZ-MP Treated Process water(final effluent)
- Final effluent mixed with Drizzle Lake water
- Drizzle Lake water.

In providing this new Table 1 it should be noted that all of the metals parameter concentrations included in this table are comparable to or lower than the parameter concentrations previously provided in Table 6.4-1 of the DAR and Table 20 in Avalon's response to EC IR #13.1.

It should also be noted that this table is more extensive than any of the other tables previously provided and it includes total and dissolved concentrations of all of the rare earth elements associated with the mineral deposit.

Table 1: Solution Analyses

Parameter	Unit	MMER 2002	Guidelines for the Protection of Aquatic Life		PP-22 BZ-MP Treated Process Water-2		PP-22 BZ-MP Treated Process + Lake Water		Lake Water	
			Short Term	Long Term						
Radionuclides										
^{226}Ra	Bq/L	0.37			pending	---	pending	---	pending	---
^{228}Ra	Bq/L				pending	---	pending	---	pending	---
^{210}Pb	Bq/L				pending	---	pending	---	pending	---
LIMS					11276-APR12		11276-APR12		11276-APR12	
Temp on Rec	°C				15.0	---	15.0	---	15.0	---
BOD5	mg/L	50 [1]			< 4	---	< 4	---	< 4	---
BOD5	mg/L	50 [1]			---	---	---	---	---	---
pH	units	6.0-9.5		6.0-9.5	7.97	---	8.52	---	8.45	---
Alkalinity	mg/L as $CaCO_3$				129	---	257	---	260	---
EMF	mV				220	---	168	---	195	---
Acidity	mg/L as $CaCO_3$				< 2	---	< 2	---	< 2	---
Conductivity	µS/cm				402	---	468	---	478	---
TDS	mg/L				229	---	349	---	338	---
TSS	mg/L	15.00	Max ↑ 25 mg/L from background		2	---	18	---		24
Cl	mg/L		640	120	42	---	8.9	---	8.9	---
SO_4	mg/L				6.5	---	< 0.2	---	< 0.2	---
F	mg/L			0.12	0.64	---	1.71	---	1.69	---
NO_2	as N mg/L			0.06	< 0.06	---	< 0.06	---	< 0.06	---
NO_3	as N mg/L			13	0.07	---	< 0.05	---	< 0.05	---
NO_2+NO_3	as N mg/L				0.07	---	< 0.06	---	< 0.06	---
Tot.Reactive P	mg/L	<0.004 to >0.1 based on lake type			0.30	---	< 0.03	---		

Table 1: Solution Analyses

Parameter	Unit	MMER 2002	Guidelines for the Protection of Aquatic Life		PP-22 BZ-MP Treated Process Water-2		PP-22 BZ-MP Treated Process + Lake Water		Lake Water	
			Short Term	Long Term						
TOC	mg/L				< 1.0	---	41.5	---	50.2	---
NH_3+NH_4	as N mg/L			0.20	< 0.1	---	1.4	---	1.4	---
COD	mg/L	150 [1]			< 8	---	111	---	109	---
Thiosalts	as S_2O_3 mg/L				< 10	---	< 10	---	< 10	---
Metals					Total	Dissolved	Total	Dissolved	Total	Dissolved
Hg	mg/L			0.000026	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Ag	mg/L			0.0001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
Al	mg/L			0.1	0.12	0.03	< 0.01	< 0.01	< 0.01	< 0.01
As	mg/L	0.50		0.005	0.0009	0.0010	0.0018	0.0021	0.0017	0.0022
Ba	mg/L				0.0062	0.0069	0.119	0.0815	0.115	0.0823
Be	mg/L				< 0.00002	< 0.00002	< 0.00002	< 0.00002	< 0.00002	< 0.00002
B	mg/L		29	1.5	0.0084	0.0091	0.0368	0.0345	0.0362	0.0360
Bi	mg/L				< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001	< 0.00001
Ca	mg/L				22.2	22.5	56.0	52.1	54.9	52.8
Cd	mg/L			0.000025	< 0.000003	< 0.000003	0.000040	< 0.000003	0.000029	0.000003
Co	mg/L				0.000071	0.000071	0.000206	0.000127	0.000195	0.000134
Cr	mg/L				< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
Cu	mg/L	0.30		0.002	0.0019	0.0014	0.0014	0.0018	0.0012	0.0038
Fe	mg/L			0.3	0.044	0.010	5.86	0.067	5.69	0.051
K	mg/L				30.8	28.6	3.71	3.52	3.56	3.54
Li	mg/L				0.011	0.010	0.010	0.009	0.010	0.010
Mg	mg/L				4.38	4.39	26.1	24.5	25.5	24.9
Mn	mg/L				0.117	0.125	0.810	0.380	0.790	0.387
Mo	mg/L				0.0062	0.0063	0.0024	0.0022	0.0022	0.0021
Na	mg/L				38.8	38.1	12.0	11.3	11.7	11.5

Table 1: Solution Analyses

Parameter	Unit	MMER 2002	Guidelines for the Protection of Aquatic Life		PP-22 BZ-MP Treated Process Water-2		PP-22 BZ-MP Treated Process + Lake Water		Lake Water	
			Short Term	Long Term						
Ni	mg/L	0.50		0.08	0.0020	0.0018	0.0013	0.0012	0.0011	0.0018
Pb	mg/L	0.20		0.002	0.00092	0.00015	0.00038	0.00005	0.00028	0.00010
Sb	mg/L				< 0.0002	< 0.0002	< 0.0002	< 0.0002	< 0.0002	< 0.0002
Se	mg/L			0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Si	mg/L				7.61	7.53	6.82	5.83	6.63	5.91
Sn	mg/L				0.00002	< 0.00001	0.00003	< 0.00001	0.00002	0.00011
Sr	mg/L				0.0912	0.0918	0.0999	0.0935	0.0982	0.0936
Th	mg/L				0.000017	< 0.000004	0.000030	< 0.000004	0.000021	< 0.000004
Ti	mg/L				0.0003	0.0002	0.0004	0.0002	0.0003	0.0002
Tl	mg/L			0.0008	< 0.0002	< 0.0002	< 0.0002	< 0.0002	< 0.0002	< 0.0002
U	mg/L		0.033	0.015	0.00001	0.00001	0.00015	0.00013	0.00015	0.00014
V	mg/L				0.00019	0.00013	0.00029	0.00020	0.00029	0.00035
Y	mg/L				0.00025	0.00004	0.00016	0.00002	0.00012	0.00003
Zn	mg/L	0.50		0.03	0.028	0.023	0.084	0.008	0.080	0.008
Ce	mg/L				0.00092	0.00020	0.00082	< 0.00007	0.00056	0.00023
Dy	mg/L				0.000063	0.000015	0.000037	0.000003	0.000027	0.000006
Er	mg/L				0.000022	0.000004	0.000017	0.000002	0.000011	0.000002
Eu	mg/L				0.000014	0.000005	0.000017	0.000006	0.000014	0.000007
Ga	mg/L				0.00005	0.00004	0.00002	0.00004	0.00001	0.00001
Gd	mg/L				0.00011	< 0.00005	0.00006	< 0.00005	< 0.00005	< 0.00005
Hf	mg/L				< 0.000005	< 0.000005	0.000028	< 0.000005	0.000027	< 0.000005
Ho	mg/L				0.000010	0.000002	0.000006	0.000001	0.000005	0.000001
La	mg/L				0.00041	0.00009	0.00038	< 0.00004	0.00026	0.00011
Lu	mg/L				0.000002	< 0.000001	0.000001	< 0.000001	0.000001	< 0.000001
Nb	mg/L				0.000045	0.000003	0.000089	0.000001	0.000065	0.000010

Table 1: Solution Analyses

Parameter	Unit	MMER 2002	Guidelines for the Protection of Aquatic Life		PP-22 BZ-MP Treated Process Water-2		PP-22 BZ-MP Treated Process + Lake Water		Lake Water	
			Short Term	Long Term						
Nd	mg/L				0.00049	0.00012	0.00041	< 0.00003	0.00028	0.00012
Pr	mg/L				0.00011	0.00003	0.00010	< 0.00001	0.00007	0.00003
Sc	mg/L				0.00082	0.00072	0.00082	0.00062	0.00080	0.00063
Sm	mg/L				0.00011	0.00003	0.00008	< 0.00001	0.00006	0.00002
Ta	mg/L				0.000009	0.000001	0.000020	0.000004	0.000021	0.000004
Tb	mg/L				0.000014	0.000005	0.000007	0.000006	0.000003	< 0.000001
Tm	mg/L				0.000003	< 0.000001	0.000002	0.000009	< 0.000001	< 0.000001
Yb	mg/L				0.000012	0.000002	0.000009	< 0.000002	0.000007	0.000002
Zr	mg/L				0.00007	0.00003	0.00013	0.00004	0.00012	0.00006
F2 (C10-C16)	µg/L				< 100	---	< 100	---	< 100	---
F3 (C16-C34)	µg/L				< 500	---	< 500	---	< 500	---
F4 (C34-C50)	µg/L				< 500	---	< 500	---	< 500	---
Baseline at nC50	Yes / No				YES	---	YES	---	YES	---

*Department of Justice Canada. 2002. Metal Mining Effluent Regulations, Fisheries Act SOR-2002-222.
Available Online: http://laws.justice.gc.ca/en/F-14/SOR-2002-222/119716.html

[1]World Bank Environment, Health and Safety Guidelines for Mining, 2007.

IR Clarification:	**#4 (Footnote)**
Source:	MVEIRB
To:	Avalon Rare Metals Inc.

Preamble

The Review Board notes that in the original DAR Table 6.4-2, the modeled ratio of Plant Discharge to the Thor Lake concentration after 20 years was reported as about 1408:1, but was subsequently corrected to about 10:1 in Avalon's response to MVRB IR #1.2. In Table 1 of Avalon's Response #2.1 to IR AANDC #2, the ratios of Day 5 Decant Concentrations to the Max. Predicted Concentrations Years 1-20 (in Thor Lake) are reported as about 1320:1, apparently reflecting the error in calculation that appeared in the original Table 6.4-2.

MVEIRB IR Clarification Request #4 (Footnote)

Please confirm that Table 1 of Avalon's Response #2.1 to IR AANDC #2 is correct or submit a corrected response.

Avalon IR Clarification Response #4 (Footnote)

The original DAR Table 6.4-2 should be replaced by the following revised table.

Table 6.4-2: Average Concentration of Inert Tracer in The Thor Lake System

Year of Simulation	Plant Discharge	Tailings Pond	Polishing Pond	Drizzle Lake	Murky Lake	Thor Lake
1	1.00000	0.00091	0.00026	0.00004	0.00003	<0.00001
2	1.00001	0.00160	0.00073	0.00021	0.00017	0.00001
3	1.00004	0.00215	0.00119	0.00043	0.00037	0.00004
4	1.00009	0.00260	0.00164	0.00064	0.00058	0.00009
5	1.00016	0.00299	0.00208	0.00092	0.00085	0.00016
6	1.00024	0.00331	0.00241	0.00111	0.00104	0.00024
7	1.00031	0.00360	0.00269	0.00126	0.00119	0.00031
8	1.00038	0.00386	0.00292	0.00138	0.00132	0.00038
9	1.00044	0.00408	0.00313	0.00152	0.00144	0.00044
10	1.00050	0.00423	0.00330	0.00159	0.00152	0.00050
11	1.00057	0.00437	0.00342	0.00178	0.00159	0.00057
12	1.00058	0.00455	0.00355	0.00179	0.00166	0.00058
13	1.00061	0.00466	0.00369	0.00180	0.00171	0.00061
14	1.00063	0.00477	0.00379	0.00185	0.00177	0.00063
15	1.00066	0.00485	0.00387	0.00190	0.00183	0.00066
16	1.00070	0.00492	0.00394	0.00199	0.00186	0.00070
17	1.00068	0.00500	0.00392	0.00194	0.00186	0.00068
18	1.00067	0.00500	0.00389	0.00191	0.00176	0.00067
19	1.00070	0.00504	0.00400	0.00199	0.00186	0.00070
20	1.00071	0.00508	0.00408	0.00207	0.00191	0.00071

The difference between this table and the original table is the second column 'Plant Discharge Concentration', which now includes the effect of recirculating water from Thor Lake through the plant.

The dilution ratio of 1,408 was and still is correct. However, the ratio of 10:1 stated in Avalon's Response #1.2, was stated in error. Therefore, for Table 1 in Avalon's Response #2.1 to AANDC's Information Requests, the dilution ratio that should be used to calculate the maximum predicted concentration for Year 1-20 should be 1,408.

As a result of changes to Table 6.4-2, Table 6.4-3 in the DAR report should also be replaced by the following table, as the effects of background concentration are now included.

Table 6.4-3: Maximum Metal Concentration in The Thor Lake System in Year 20 and Water Quality Guidelines for The Metals of Concern

Metal Species	Background Concentration			Thor Lake	Murky Lake	Drizzle Lake	CCME Water Quality Guideline	MMER Effluent Criteria
	Thor Lake	Murky Lake	Drizzle Lake					
Hg (mg/L)	0.000010	0.000010	0.000005	0.000010	0.000010	0.000005	0.000026	-
Ag (mg/L)	0.00001	0.00001	0.00001	0.0001	0.00001	0.00001	0.0001	-
Al (mg/L)	0.0033	0.0072	0.0083	0.0037	0.0084	0.0096	0.1	-
As (mg/L)	0.00077	0.00129	0.00092	0.00077	0.00129	0.00092	0.005	0.5
Ba (mg/L)	0.0655	0.0636	0.0629	0.0655	0.0636	0.0629	-	-
Be (mg/L)	0.00025	0.00025	0.00025	0.00025	0.00025	0.00025	-	-
B (mg/L)	0.026	0.025	0.026	0.026	0.025	0.026	-	-
Bi (mg/L)	0.00025	0.00025	0.00025	0.00025	0.00025	0.00025	-	-
Ca (mg/L)	35.1	36.6	30.8	35.1	36.7	30.9	-	-
Cd (mg/L)	0.00002	0.00002	0.00001	0.00002	0.00002	0.00001	0.00002 - 0.00013	-
Co (mg/L)	0.00005	0.00009	0.00017	0.00005	0.00009	0.00017	-	-
Cr (mg/L)	0.00028	0.00025	0.00025	0.00028	0.00025	0.00025	0.0089	-
Cu (mg/L)	0.00036	0.00036	0.00025	0.00036	0.00036	0.00025	0.002 -0.004	0.3
Fe (mg/L)	0.070	3.054	1.091	0.070	3.055	1.092	0.3	-
K (mg/L)	2.0	1.8	1.3	2.0	1.9	1.4	-	-
Li (mg/L)	0.0054	0.0051	0.0041	0.0054	0.0052	0.0042	-	-
Mg (mg/L)	18.8	17.8	15.4	18.8	17.8	15.4	-	-
Mn (mg/L)	0.0414	0.2476	0.1872	0.0415	0.2478	0.1874	-	-
Mo (mg/L)	0.00210	0.00142	0.00127	0.00213	0.00151	0.00137	0.073	-
Na (mg/L)	6.8	7.1	6.1	6.8	7.2	6.2	-	-
Ni (mg/L)	0.00025	0.00025	0.00025	0.00025	0.00026	0.00026	0.025 -0.150	0.5
Pb (mg/L)	0.000050	0.000030	0.000028	0.000050	0.000031	0.000029	0.001 -0.007	0.2
Sb (mg/L)	0.00005	0.00005	0.00005	0.00005	0.00006	0.00006	-	-
Se (mg/L)	0.0005	0.0005	0.0005	0.0005	0.00005	0.00005	0.001	-
Si (mg/L)	3.28	2.17	2.37	3.28	2.19	2.39	-	-

Table 6.4-3: Maximum Metal Concentration in The Thor Lake System in Year 20 and Water Quality Guidelines for The Metals of Concern

Sn (mg/L)	0.00008	0.00005	0.00008	0.00008	0.00005	0.00008	-	-
Sr (mg/L)	0.0581	0.0562	0.0523	0.0582	0.0567	0.0528	-	-
Ti (mg/L)	0.005	0.005	0.005	0.005	0.005	0.005	-	-
Tl (mg/L)	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.0008	-
U (mg/L)	0.000357	0.000098	0.000077	0.000363	0.000115	0.000095	0.015	-
V (mg/L)	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	-	-
Zn (mg/L)	0.0014	0.0023	0.0009	0.0014	0.0023	0.0009	0.03	0.5

ATTACHMENTS

Attachment 1: Acute and chronic bioassay test results on simulated Flotation plant TMF effluent, effluent/Drizzle Lake mix, and Drizzle Lake water

Attachment 2: EBA, A Tetra Tech Company (EBA). April 18, 2012. Background Water Quality Assessment – Avalon Thor Lake Project. Technical Memo.

Attachment 3: EBA, A Tetra Tech Company (EBA). May 1, 2012. April 20, 2012 Meeting Notes: Avalon Thor Lake Site Specific Water Quality Objectives.

Attachment 4: Ng, T., D. S. Smith, A. Straus, and J. C. McGeer. March 31, 2011. Review of Aquatic Effects of Lanthanides and Other Uncommon Elements. Final Project Report prepared for the EC Contribution Agreement with the CNTC for 2010/2011 by Wilfrid Laurier University.

May 10, 2012

Attachment 1

AVALON RARE METALS INC.

May 10, 2012

Acute Bioassay Test Results: Rainbow Trout

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221256
Sample Number : 33957

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited	Time Collected :	15:50
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process Water-2	Date Received :	2012-04-24
Sampling Method :	Grab	Date Tested :	2012-04-25
Sampled By :	B. Bowman	Temp. on arrival :	13.0 °C
Sample Description :	Clear, colourless, odourless.		

Test Method : Reference Method for Determining Acute Lethality of Liquid Effluents to Rainbow Trout. Environment Canada, EPS 1/RM/13 (2nd Edition, December 2000, with May 2007 amendments).

TEST RESULTS

Effect	Value	95% Confidence Limits	Slope	Calculation Method
96-h LC50	>100%	-	-	-

The results reported relate only to the sample tested.

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Organism Batch :	T12-04		
Date Tested (yyyy-mm-dd) :	2012-04-19	Historical Mean LC50 :	3817 mg/L
LC50 (95% Confidence Limits) :	3975 mg/L (3644 - 4336)	Warning Limits (± 2SD) :	3245 - 4489 mg/L
Statistical Method :	Spearman-Kärber	Analyst(s) :	FS/CN/DK/JGG

TEST FISH

Control Fish Sample Size :	10	Cumulative stock tank mortality :	0 % (prev. 7 days)
Mean Fish Weight (± 2 SD) :	0.70 ± 0.12 g	Mean Fish Fork Length (± 2 SD) :	42.0 ± 3.3 mm
Range of Weights :	0.64 - 0.82 g	Range of Fork Lengths :	40 - 45 mm
Fish Loading Rate :	0.4 g/L		

TEST CONDITIONS

Test Organism :	*Oncorhynchus mykiss*	Volume Tested (L) :	16
Sample Treatment :	None	Number of Replicates :	1
pH Adjustment :	None	Organisms Per Replicate :	10
Test Aeration :	Yes	Total Organisms Per Test Level :	10
Pre-aeration/Aeration Rate :	6.5 ± 1 mL/min/L	Test Method Deviation(s) :	None

Date: 2012-05-03
yyyy-mm-dd

Approved by: [signature]
Project Manager

Work Order: 221256
Sample Number: 33957

Total Pre-Aeration Time (h)		pH	D.O. (mg/L)	Cond. (µmhos/cm)	Temp. (°C)	O_2 Sat. (%)*
	Initial Water Chemistry:	7.4	7.9	396	15.5	–
0:30	Chemistry after 30min air:	7.6	8.4	401	15.0	87

0 hours

Date & Time 2012-04-25 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.	O_2 Sat. (%)*
100	0	0	7.6	8.4	401	15.0	87
50	0	0	8.0	9.0	615	14.5	
25	0	0	8.1	9.2	690	14.5	
12.5	0	0	8.2	9.4	735	14.5	
6.25	0	0	8.2	9.4	755	14.5	
Control	0	0	8.3	9.7	772	14.5	100

Notes:

24 hours

Date & Time 2012-04-26 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	15.0
50	0	0	–	–	–	15.0
25	0	0	–	–	–	15.0
12.5	0	0	–	–	–	15.0
6.25	0	0	–	–	–	15.0
Control	0	0	–	–	–	15.0

Notes:

48 hours

Date & Time 2012-04-27 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	14.5
50	0	0	–	–	–	14.5
25	0	0	–	–	–	14.5
12.5	0	0	–	–	–	14.5
6.25	0	0	–	–	–	14.5
Control	0	0	–	–	–	14.5

Notes:

72 hours

Date & Time 2012-04-28 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	14.5
50	0	0	–	–	–	14.5
25	0	0	–	–	–	14.5
12.5	0	0	–	–	–	14.5
6.25	0	0	–	–	–	14.5
Control	0	0	–	–	–	14.5

Notes:

96 hours

Date & Time 2012-04-29 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	8.4	9.1	406	14.0
50	0	0	8.4	8.9	626	14.0
25	0	0	8.4	9.2	705	14.0
12.5	0	0	8.4	9.0	722	14.0
6.25	0	0	8.4	9.1	730	14.0
Control	0	0	8.3	9.2	740	14.0

Notes:

# of control organisms showing stress: 0
Trout Batch #: T12-04

Number immobile does not include number of mortalities.
* adjusted for actual temp. & barometric pressure
"–" = not measured

Test Data Reviewed By: [illegible]
Date: [illegible]

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

TOXICITY TEST REPORT
Rainbow Trout

Work Order : 221256
Sample Number : 33958

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited	Time Collected :	15:50
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process + Lake Water	Date Received :	2012-04-24
Sampling Method :	Grab	Date Tested :	2012-04-25
Sampled By :	B. Bowman	Temp. on arrival :	13.0 °C
Sample Description :	Clear, orange, odourless.		

Test Method : Reference Method for Determining Acute Lethality of Liquid Effluents to Rainbow Trout. Environment Canada, EPS 1/RM/13 (2nd Edition, December 2000, with May 2007 amendments).

TEST RESULTS

Effect	Value	95% Confidence Limits	Slope	Calculation Method
96-h LC50	>100%	-	-	-

The results reported relate only to the sample tested.

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Organism Batch :	T12-04		
Date Tested (yyyy-mm-dd) :	2012-04-19	Historical Mean LC50 :	3817 mg/L
LC50 (95% Confidence Limits) :	3975 mg/L (3644 - 4336)	Warning Limits (± 2SD) :	3245 - 4489 mg/L
Statistical Method :	Spearman-Kärber	Analyst(s) :	FS/CN/DK/JGG

TEST FISH

Control Fish Sample Size :	10	Cumulative stock tank mortality :	0 % (prev. 7 days)
Mean Fish Weight (± 2 SD) :	0.42 ± 0.21 g	Mean Fish Fork Length (± 2 SD) :	36.3 ± 6.1 mm
Range of Weights :	0.31 - 0.61 g	Range of Fork Lengths :	32 - 41 mm
Fish Loading Rate :	0.4 g/L		

TEST CONDITIONS

Test Organism :	*Oncorhynchus mykiss*	Volume Tested (L) :	10
Sample Treatment :	None	Number of Replicates :	1
pH Adjustment :	None	Organisms Per Replicate :	10
Test Aeration :	Yes	Total Organisms Per Test Level :	10
Pre-aeration/Aeration Rate :	6.5 ± 1 mL/min/L	Test Method Deviation(s) :	None

Date: 2012-05-03
yyyy-mm-dd

Approved by: [signature]
Project Manager

AQUATOX

Work Order: 221256
Sample Number: 33958

Total Pre-Aeration Time (h)		pH	D.O. (mg/L)	Cond. (μmhos/cm)	Temp. (°C)	O_2 Sat. (%)*
	Initial Water Chemistry:	8.4	8.3	479	15.0	–
0:30	Chemistry after 30min air:	8.5	8.9	481	14.5	93

0 hours

Date & Time 2012-04-25 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.	O_2 Sat. (%)*
100	0	0	8.5	8.9	481	14.5	93
50	0	0	8.4	9.2	646	14.5	
25	0	0	8.3	9.3	711	14.5	
12.5	0	0	8.2	9.4	745	14.5	
6.25	0	0	8.2	9.5	753	14.5	
Control	0	0	8.3	9.7	772	14.5	100

Notes:

24 hours

Date & Time 2012-04-26 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	15.0
50	0	0	–	–	–	15.0
25	0	0	–	–	–	15.0
12.5	0	0	–	–	–	15.0
6.25	0	0	–	–	–	15.0
Control	0	0	–	–	–	15.0

Notes:

48 hours

Date & Time 2012-04-27 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	14.5
50	0	0	–	–	–	14.5
25	0	0	–	–	–	14.5
12.5	0	0	–	–	–	14.5
6.25	0	0	–	–	–	14.5
Control	0	0	–	–	–	14.5

Notes:

72 hours

Date & Time 2012-04-28 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	14.5
50	0	0	–	–	–	14.5
25	0	0	–	–	–	14.5
12.5	0	0	–	–	–	14.5
6.25	0	0	–	–	–	14.5
Control	0	0	–	–	–	14.5

Notes:.

96 hours

Date & Time 2012-04-29 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	8.5	9.2	491	14.0
50	0	0	8.5	9.0	650	14.0
25	0	0	8.5	9.1	733	14.0
12.5	0	0	8.4	8.9	763	14.0
6.25	0	0	8.4	8.9	776	14.0
Control	0	0	8.3	9.0	779	14.0

Notes:

# of control organisms showing stress: 0
Trout Batch #: T12-04

Number immobile does not include number of mortalities.
* adjusted for actual temp. & barometric pressure
"–" = not measured

Test Data Reviewed By: [signature]
Date: 2012-04-30

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

TOXICITY TEST REPORT
Rainbow Trout

Work Order :	221256
Sample Number :	33959

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited	Time Collected :	15:50
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	Lake Water	Date Received :	2012-04-24
Sampling Method :	Grab	Date Tested :	2012-04-25
Sampled By :	B. Bowman	Temp. on arrival :	13.0 °C
Sample Description :	Cloudy, brown, odourless.		

Test Method : Reference Method for Determining Acute Lethality of Liquid Effluents to Rainbow Trout. Environment Canada, EPS 1/RM/13 (2nd Edition, December 2000, with May 2007 amendments).

TEST RESULTS

Effect	Value	95% Confidence Limits	Slope	Calculation Method
96-h LC50	>100%	-	-	-

The results reported relate only to the sample tested.

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Organism Batch :	T12-04		
Date Tested (yyyy-mm-dd) :	2012-04-19	Historical Mean LC50 :	3817 mg/L
LC50 (95% Confidence Limits) :	3975 mg/L (3644 - 4336)	Warning Limits (± 2SD) :	3245 - 4489 mg/L
Statistical Method :	Spearman-Kärber	Analyst(s) :	FS/CN/DK/JGG

TEST FISH

Control Fish Sample Size :	10	Cumulative stock tank mortality :	0 % (prev. 7 days)
Mean Fish Weight (± 2 SD) :	0.47 ± 0.21 g	Mean Fish Fork Length (± 2 SD) :	37.6 ± 4.5 mm
Range of Weights :	0.34 - 0.62 g	Range of Fork Lengths :	34 - 40 mm
Fish Loading Rate :	0.4 g/L		

TEST CONDITIONS

Test Organism :	*Oncorhynchus mykiss*	Volume Tested (L) :	12
Sample Treatment :	None	Number of Replicates :	1
pH Adjustment :	None	Organisms Per Replicate :	10
Test Aeration :	Yes	Total Organisms Per Test Level :	10
Pre-aeration/Aeration Rate :	6.5 ± 1 mL/min/L	Test Method Deviation(s) :	None

Date: 2012-05-03
yyyy-mm-dd

Approved by: [signature]
Project Manager

Work Order: 221256
Sample Number: 33959

Total Pre-Aeration Time (h)		pH	D.O. (mg/L)	Cond. (µmhos/cm)	Temp. (°C)	O_2 Sat. (%)*
	Initial Water Chemistry:	8.5	8.7	479	15.0	–
0:30	Chemistry after 30min air:	8.6	9.3	481	15.0	96

0 hours

Date & Time 2012-04-25 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.	O_2 Sat. (%)*
100	0	0	8.6	9.3	481	15.0	96
50	0	0	8.4	9.3	644	14.5	
25	0	0	8.4	9.3	721	14.5	
12.5	0	0	8.3	9.3	752	14.5	
6.25	0	0	8.4	9.3	759	14.5	
Control	0	0	8.3	9.7	772	14.5	100

Notes:

24 hours

Date & Time 2012-04-26 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	15.0
50	0	0	–	–	–	15.0
25	0	0	–	–	–	15.0
12.5	0	0	–	–	–	15.0
6.25	0	0	–	–	–	15.0
Control	0	0	–	–	–	15.0

Notes:

48 hours

Date & Time 2012-04-27 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	14.5
50	0	0	–	–	–	14.5
25	0	0	–	–	–	14.5
12.5	0	0	–	–	–	14.5
6.25	0	0	–	–	–	14.5
Control	0	0	–	–	–	14.5

Notes:

72 hours

Date & Time 2012-04-28 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	14.5
50	0	0	–	–	–	14.5
25	0	0	–	–	–	14.5
12.5	0	0	–	–	–	14.5
6.25	0	0	–	–	–	14.5
Control	0	0	–	–	–	14.5

Notes:

96 hours

Date & Time 2012-04-29 10:10
Technician: FS

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	8.5	8.3	490	14.0
50	0	0	8.5	8.4	651	14.0
25	0	0	8.4	8.5	715	14.0
12.5	0	0	8.4	8.6	753	14.0
6.25	0	0	8.4	8.6	763	14.0
Control	0	0	8.3	9.0	770	14.0

Notes:

# of control organisms showing stress: 0
Trout Batch #: T12-04

Number immobile does not include number of mortalities.
* adjusted for actual temp. & barometric pressure
"–" = not measured

Test Data Reviewed By: [illegible]
Date: 2012-04-30

May 10, 2012

Acute Bioassay Test Results:

Daphnia Magna

AQUATOX

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

TOXICITY TEST REPORT

Daphnia magna

Work Order : 221256
Sample Number : 33957

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited	Time Collected :	15:50
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process Water-2	Date Received :	2012-04-24
Sampling Method :	Grab	Date Tested :	2012-04-24
Sampled By :	B. Bowman	Temp. on arrival :	13.0° C
Sample Description :	Clear, colourless, odourless.		

Test Method : Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna.* Environment Canada EPS 1/RM/14 (Second Edition, December 2000).

TEST RESULTS

Effect	Value	95% Confidence Limits	Slope	Calculation Method
48-h LC50	>100%	-	-	-

The results reported relate only to the sample tested.

SODIUM CHLORIDE REFERENCE TOXICANT DATA

Organism Batch :	Dm12-07		
Date Tested (yyyy/mm/dd) :	2012-04-17	Historical Mean LC50 :	6.4 g/L
LC50 (95% Confidence Limits) :	6.9 g/L (6.6 - 7.3)	Warning Limits (± 2SD) :	5.9 - 7.0 g/L
Statistical Method :	Spearman-Kärber	Analyst(s) :	NK

Daphnia magna CULTURE HEALTH DATA

Time to First Brood :	9.2 days	Mean Young Per Brood :	29.7
Culture Mortality :	0% (previous 7 days)		

TEST CONDITIONS

Sample Treatment :	None	Number of Replicates :	1
pH Adjustment :	None	Test Organisms / Replicate :	10
Test Aeration :	None	Total Organisms / Test Level :	10
Organism Batch :	Dm12-07	Organism Loading Rate :	15.0 mL/organism
		Test Method Deviation(s) :	None

Date: 2012-05-03
yyyy-mm-dd

Approved by: [signature]
Project Manager

AQUATOX

Work Order: 221256
Sample Number: 33957

	Hardness (mg/L as $CaCO_3$)	Hardness Adjustment	pH	D.O. (mg/L)	Cond. (μmhos/cm)	Temp. (°C)	O_2 Sat. (%)*	Total Pre-Aeration Time (h) @ 30 mL/min/L
Initial Water Chemistry:	90	None	7.5	7.9	423	21.0	93	0:00

0 hours

Date & Time 2012-04-24 14:20
Technician: AW

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.	O_2 Sat. (%)*	Hardness
100	0	0	7.5	7.9	423	21.0	93	90
50	0	0	7.9	8.3	436	21.0		
25	0	0	8.1	8.3	442	21.0		
12.5	0	0	8.3	8.3	444	21.0		
6.25	0	0	8.3	8.2	445	21.0		
Control	0	0	8.3	8.2	445	21.0	97	200

Notes:

24 hours

Date & Time 2012-04-25 14:20
Technician: NK

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	1	–	–	–	20.5
50	0	0	–	–	–	20.5
25	0	0	–	–	–	20.5
12.5	0	0	–	–	–	20.5
6.25	0	0	–	–	–	20.5
Control	0	0	–	–	–	20.5

Notes:

48 hours

Date & Time 2012-04-26 14:20
Technician: AW

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	1	8.5	8.4	424	21.0
50	0	0	8.5	8.5	440	21.0
25	0	0	8.5	8.4	446	21.0
12.5	0	0	8.5	8.5	447	21.0
6.25	0	0	8.5	8.4	450	21.0
Control	0	0	8.5	8.5	462	21.0

Notes:

# of control organisms showing stress: 0
Daphnia Batch #: Dm12-07

Number immobile does not include number of mortalities.

– = not measured
* adjusted for actual temp. & barometric pressure

Test Data Reviewed By: [illegible]
Date: [illegible]

AquaTox Testing & Consulting Inc.
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Tel: (519) 763-4412 Fax: (519) 763-4419

TOXICITY TEST REPORT
Daphnia magna

Work Order : 221256
Sample Number : 33958

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited	Time Collected :	15:50
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process + Lake Water	Date Received :	2012-04-24
Sampling Method :	Grab	Date Tested :	2012-04-24
Sampled By :	B. Bowman	Temp. on arrival :	13.0° C
Sample Description :	Clear, orange, odourless.		

Test Method : Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna.* Environment Canada EPS 1/RM/14 (Second Edition, December 2000).

TEST RESULTS

Effect	Value	95% Confidence Limits	Slope	Calculation Method
48-h LC50	>100%	-	-	-

The results reported relate only to the sample tested.

SODIUM CHLORIDE REFERENCE TOXICANT DATA

Organism Batch :	Dm12-07		
Date Tested (yyyy/mm/dd) :	2012-04-17	Historical Mean LC50 :	6.4 g/L
LC50 (95% Confidence Limits) :	6.9 g/L (6.6 - 7.3)	Warning Limits (± 2SD) :	5.9 - 7.0 g/L
Statistical Method :	Spearman-Kärber	Analyst(s) :	NK

Daphnia magna CULTURE HEALTH DATA

Time to First Brood :	9.2 days	Mean Young Per Brood :	29.7
Culture Mortality :	0% (previous 7 days)		

TEST CONDITIONS

Sample Treatment :	None	Number of Replicates :	1
pH Adjustment :	None	Test Organisms / Replicate :	10
Test Aeration :	None	Total Organisms / Test Level :	10
Organism Batch :	Dm12-07	Organism Loading Rate :	15.0 mL/organism
		Test Method Deviation(s) :	None

Date: 2012-05-03
yyyy-mm-dd

Approved by: [signature]
Project Manager

Work Order: 221256
Sample Number: 33958

	Hardness (mg/L as $CaCO_3$)	Hardness Adjustment	pH	D.O. (mg/L)	Cond. (µmhos/cm)	Temp. (°C)	O_2 Sat. (%)*	Total Pre-Aeration Time (h) @ 30 mL/min/L
Initial Water Chemistry:	250	None	8.5	9.1	499	21.0	106	0:30

0 hours

Date & Time 2012-04-24 14:35
Technician: AW

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.	O_2 Sat. (%)*	Hardness
100	0	0	8.6	8.6	498	21.0	99	250
50	0	0	8.5	8.4	474	21.0		
25	0	0	8.5	8.3	461	21.0		
12.5	0	0	8.4	8.4	454	21.0		
6.25	0	0	8.4	8.3	451	21.0		
Control	0	0	8.3	8.2	445	21.0	97	200

Notes:

24 hours

Date & Time 2012-04-25 14:35
Technician: NK

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	20.5
50	0	0	–	–	–	20.5
25	0	0	–	–	–	20.5
12.5	0	0	–	–	–	20.5
6.25	0	0	–	–	–	20.5
Control	0	0	–	–	–	20.5

Notes:

48 hours

Date & Time 2012-04-26 14:35
Technician: AW

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	8.7	8.3	501	21.0
50	0	0	8.6	8.4	475	21.0
25	0	0	8.6	8.6	463	21.0
12.5	0	0	8.6	8.5	459	21.0
6.25	0	0	8.5	8.4	455	21.0
Control	0	0	8.5	8.5	454	21.0

Notes:

# of control organisms showing stress: 0
Daphnia Batch #: Dm12-07

Number immobile does not include number of mortalities.

– = not measured
* adjusted for actual temp. & barometric pressure

Test Data Reviewed By: ______
Date: ______

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TOXICITY TEST REPORT

Daphnia magna

Work Order : 221256
Sample Number : 33959

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited	Time Collected :	15:50
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	Lake Water	Date Received :	2012-04-24
Sampling Method :	Grab	Date Tested :	2012-04-24
Sampled By :	B. Bowman	Temp. on arrival :	13.0° C
Sample Description :	Cloudy, brown, odourless.		

Test Method : Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna.* Environment Canada EPS 1/RM/14 (Second Edition, December 2000).

TEST RESULTS

Effect	Value	95% Confidence Limits	Slope	Calculation Method
48-h LC50	>100%	-	-	-

The results reported relate only to the sample tested.

SODIUM CHLORIDE REFERENCE TOXICANT DATA

Organism Batch :	Dm12-07		
Date Tested (yyyy/mm/dd) :	2012-04-17	Historical Mean LC50 :	6.4 g/L
LC50 (95% Confidence Limits) :	6.9 g/L (6.6 - 7.3)	Warning Limits (± 2SD) :	5.9 - 7.0 g/L
Statistical Method :	Spearman-Kärber	Analyst(s) :	NK

Daphnia magna CULTURE HEALTH DATA

Time to First Brood :	9.2 days	Mean Young Per Brood :	29.7
Culture Mortality :	0% (previous 7 days)		

TEST CONDITIONS

Sample Treatment :	None	Number of Replicates :	1
pH Adjustment :	None	Test Organisms / Replicate :	10
Test Aeration :	None	Total Organisms / Test Level :	10
Organism Batch :	Dm12-07	Organism Loading Rate :	15.0 mL/organism
		Test Method Deviation(s) :	None

Date: 2012-05-03
yyyy-mm-dd

Approved by: [signature]
Project Manager

Work Order: 221256
Sample Number: 33959

	Hardness (mg/L as $CaCO_3$)	Hardness Adjustment	pH	D.O. (mg/L)	Cond. (µmhos/cm)	Temp. (°C)	O_2 Sat. (%)*	Total Pre-Aeration Time (h) @ 30 mL/min/L
Initial Water Chemistry:	250	None	8.6	9.1	498	21.0	105	0:30

0 hours

Date & Time 2012-04-24 14:50
Technician: AW

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.	O_2 Sat. (%)*	Hardness
100	0	0	8.6	8.6	498	21.0	100	250
50	0	0	8.5	8.4	466	21.0		
25	0	0	8.5	8.3	460	21.0		
12.5	0	0	8.5	8.3	456	21.0		
6.25	0	0	8.5	8.2	454	21.0		
Control	0	0	8.3	8.2	445	21.0	97	200

Notes:

24 hours

Date & Time 2012-04-25 14:50
Technician: NK

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	–	–	–	20.5
50	0	0	–	–	–	20.5
25	0	0	–	–	–	20.5
12.5	0	0	–	–	–	20.5
6.25	0	0	–	–	–	20.5
Control	0	0	–	–	–	20.5

Notes:

48 hours

Date & Time 2012-04-26 14:50
Technician: AW

Test Conc. (%)	Mortality	Immobility	pH	D.O.	Cond.	Temp.
100	0	0	8.7	8.3	502	21.0
50	0	0	8.6	8.5	476	21.0
25	0	0	8.6	8.5	463	21.0
12.5	0	0	8.5	8.4	458	21.0
6.25	0	0	8.5	8.5	452	21.0
Control	0	0	8.5	8.5	450	21.0

Notes:

# of control organisms showing stress: 0
Daphnia Batch #: Dm12-07

Number immobile does not include number of mortalities.

– = not measured
* adjusted for actual temp. & barometric pressure

Test Data Reviewed By: ______
Date: 2012-04-30

May 10, 2012

Chronic Bioassay Test Results:
Fathead Minnow

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Larval Fathead Minnow Test Report

Survival and Growth

Work Order : 221257
Sample Number : 33960

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process Water-2	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-24
Sample Description :	Clear, colourless, odourless.		
Test Method :	Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/22 , February 1992 (including Nov. 1997 and Sept. 2008 amendments).		

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth from Biomass)	>100%	-	-
LC50	>100%	-	-

The results reported relate only to the sample tested.

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	2012-04-25	Analyst(s) :	RD/MR/CL
Organism Batch :	Fm12-04	Test Duration :	7 days
IC25 Growth (from Biomass) :	0.99 g/L	LC50 :	1.09 g/L
95% Confidence Limits :	0.83 - 1.05 g/L	95% Confidence Limits :	0.87 - 1.36 g/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Statistical Method :	Probit (Stephan)[c]
Historical Mean IC25 :	0.97 g/L	Historical Mean LC50 :	1.12 g/L
Warning Limits (± 2SD) :	0.82 - 1.14 g/L	Warning Limits (± 2SD) :	1.00 - 1.25 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

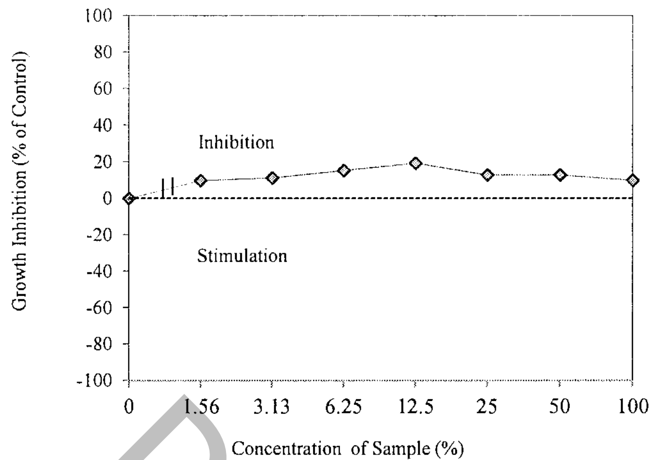
Test Organism :	*Pimephales promelas*	Test Type :	Static Renewal
Organism Batch :	Fm12-04	Control/Dilution Water :	Well water (no chemicals added)
Organism Age :	~07:00 - 23:45 h at start of test	Test Volume / Replicate :	300 mL
Source :	In-house culture	Test Vessel :	420 mL polystyrene beaker
Culture Mortality/Diseased :	0.07 % (previous 7 days)	Depth of Test Solution :	8 cm
pH Adjustment :	None	Organisms per Replicate :	10
Sample Filtration :	None	Number of Replicates :	3
Hardness Adjustment :	None	Daily Renewal Method :	80-85% syphoned and replaced
Test Aeration :	None	Test Method Deviation(s):	None

COMMENTS

- All test validity criteria as specified in the test method cited above were satisfied.
- No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.
- Inflated swim bladders were confirmed in all test organisms used in this test.

Work Order : 221257
Sample Number : 33960

Fathead Minnow Growth Inhibition (based on Biomass)



REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

[c] Stephan, C. E. 1977. Methods for calculating an LC50. pp 65-84 in : P. L. Mayer and J. L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation. Amer. Soc. Testing and Materials, Philadelphia PA. ASTM STP 634.

Date : ______________________ ***Approved By :*** ______________________

yyyy-mm-dd *Project Manager*

AQUATOX

Work Order : 221257
Sample Number : 33960

CUMULATIVE DAILY CONTROL MORTALITY AND IMPAIRMENT (±SD)

Date :	2012-04-24	2012-04-25	2012-04-26	2012-04-27	2012-04-28	2012-04-29	2012-04-30	2012-05-01
	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)

FATHEAD MINNOW CUMULATIVE DAILY MORTALITY

Initiation Time : 14:15
Initiation Date : 2012-04-24
Completion Date : 2012-05-01

		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Treatment Mean Mortality (± SD)
Date :		2012-04-24		2012-04-25		2012-04-26		2012-04-27		2012-04-28		2012-04-29		2012-04-30		2012-05-01		
Analyst(s):		XD		XD		XD		RD		FS		FS		XD		DK		
Concentration (%)	**Replicate**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	%
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
Control	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
1.56	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
3.13	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
6.25	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
12.5	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
25	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
50	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
100	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	

Aberrant behaviour or swimming impairment : None

Data Reviewed By: ______

Date : 2012-05-03

Work Order : 221257
Sample Number : 33960

FATHEAD MINNOW DRY WEIGHT AND BIOMASS DATA

Concentration (%)	Replicate	Number of Larvae Exposed	Replicate Mean Dry Weight (mg)	Treatment Mean Biomass (mg)	Standard Deviation
	A	10	0.985		
Control	B	10	0.936	0.934	0.052
	C	10	0.881		
	A	10	0.810		
1.56	B	10	0.803	0.842	0.062
	C	10	0.914		
	A	10	0.879		
3.13	B	10	0.792	0.830	0.045
	C	10	0.818		
	A	10	0.779		
6.25	B	10	0.790	0.791	0.013
	C	10	0.805		
	A	10	0.743		
12.5	B	10	0.694	0.755	0.068
	C	10	0.828		
	A	10	0.817		
25	B	10	0.782	0.816	0.033
	C	10	0.848		
	A	10	0.871		
50	B	10	0.783	0.814	0.049
	C	10	0.789		
	A	10	0.807		
100	B	10	0.821	0.843	0.050
	C	10	0.900		

NOTES :
- No outlying data points were detected according to Grubbs Test [b].
- Control average dry weight per surviving organism = 0.934 mg

Data Reviewed By: ______

Date : 2012-05-03

AQUATOX

Work Order : 221257
Sample Number: 33960

Fathead Minnow Water Chemistry Data

Initial Chemistry:	Temp. (°C)	DO (mg/L)	pH	Conductivity (µmhos/cm)	Hardness (mg/L as $CaCO_3$)
	25.0	7.7	7.6	421	90

		Day 0 - 1 2012-04-24	Day 1 - 2 2012-04-25	Day 2 - 3 2012-04-26	Day 3 - 4 2012-04-27	Day 4 - 5 2012-04-28	Day 5 - 6 2012-04-29	Day 6 - 7 2012-04-30
Sub-sample Used		1	1	1	2	2	3	3
Temperature (°C)		25.0	24.0	24.0	24.0	24.0	24.0	24.0
Dissolved Oxygen (mg/L)		7.7	7.2	8.2	7.7	8.1	8.5	8.7
Dissolved Oxygen % Sat.[1]		100	91	103	95	99	104	108
pH		7.6	7.6	7.6	7.6	7.6	7.7	7.7
Pre-aeration Time (min)[2]		0	0	20	0	0	20	20
Analyst(s) :	Initial	CL	CL	AW	AW	JGG	CL	AW
	Final	CL	CL	AW	MR	CL	AW	HL(RD)
Control (0%)								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.5	24.0	24.0	24.0	24.5
DO % Sat.	Initial	95	89	98	95	97	100	97
DO (mg/L)	Initial	7.6	7.2	7.8	7.8	8.0	8.2	8.1
	Final	7.5	7.1	6.3	6.8	6.4	6.8	6.6
pH	Initial	8.3	8.3	8.4	8.2	8.3	8.4	8.3
	Final	8.2	8.1	8.1	8.0	7.9	8.0	7.7
Cond. (µmhos)	Initial	501	499	555	483	481	476	479
1.56 %								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.5	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.6	7.8	7.9	8.0	8.3	8.2	8.1
	Final	7.4	7.0	6.3	6.7	5.7	6.8	6.8
pH	Initial	8.4	8.3	8.4	8.3	8.4	8.4	8.3
	Final	8.2	8.1	8.0	8.0	7.8	8.0	7.8
Cond. (µmhos)	Initial	502	501	485	482	481	477	475
25 %								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.5	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.7	7.8	7.9	8.1	8.0	8.3	8.2
	Final	7.6	7.1	6.0	7.1	6.3	7.0	5.9
pH	Initial	8.2	8.2	8.3	8.2	8.3	8.3	8.3
	Final	8.2	8.1	7.9	8.0	7.9	8.0	7.8
Cond. (µmhos)	Initial	485	484	472	471	454	462	460
100 %								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.5	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.6	7.7	8.0	7.9	8.3	8.4	8.4
	Final	7.3	7.1	5.9	7.1	6.2	7.2	6.4
pH	Initial	7.8	7.9	8.0	7.9	8.0	8.1	8.0
	Final	8.1	8.1	7.9	8.0	7.9	8.1	7.8
Cond. (µmhos)	Initial	425	426	423	427	429	405	405

"--" = not measured

[1] % saturation (adjusted for actual temperature and barometric pressure)

[2] ≤100 bubbles/minute

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Larval Fathead Minnow Test Report

Survival and Growth

Work Order : 221257
Sample Number : 33961

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process + Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-24
Sample Description :	Clear, orange, odourless		
Test Method :	Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/22 , 2nd ed. (February 2011).		

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth from Biomass)	>100%	-	-
LC50	>100%	-	-

The results reported relate only to the sample tested.

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	2012-04-25	Analyst(s) :	RD/MR/CL
Organism Batch :	Fm12-04	Test Duration :	7 days
IC25 Growth (from Biomass) :	0.99 g/L	LC50 :	1.09 g/L
95% Confidence Limits :	0.83 - 1.05 g/L	95% Confidence Limits :	0.87 - 1.36 g/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Statistical Method :	Probit (Stephan)[c]
Historical Mean IC25 :	0.97 g/L	Historical Mean LC50 :	1.12 g/L
Warning Limits (± 2SD) :	0.82 - 1.14 g/L	Warning Limits (± 2SD) :	1.00 - 1.25 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

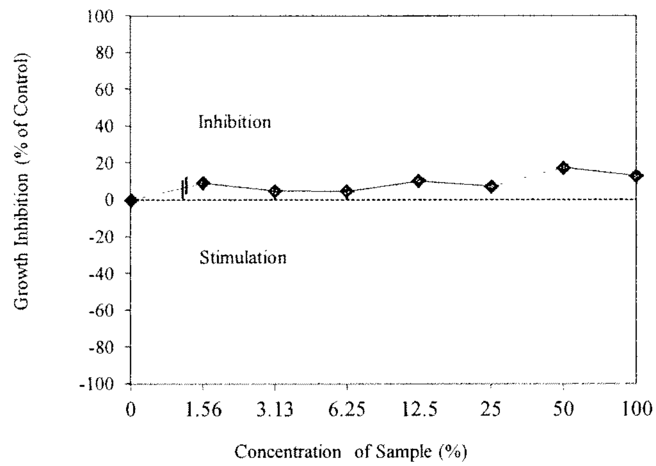
Test Organism :	*Pimephales promelas*	Test Type :	Static Renewal
Organism Batch :	Fm12-04	Control/Dilution Water :	Well water (no chemicals added)
Organism Age :	~07:00 - 23:30 h at start of test	Test Volume / Replicate :	300 mL
Source :	In-house culture	Test Vessel :	420 mL polystyrene beaker
Culture Mortality/Diseased :	0.07 % (previous 7 days)	Depth of Test Solution :	8 cm
pH Adjustment :	None	Organisms per Replicate :	10
Sample Filtration :	None	Number of Replicates :	3
Hardness Adjustment :	None	Daily Renewal Method :	80-85% syphoned and replaced
Test Aeration :	None	Test Method Deviation(s):	None

COMMENTS

- All test validity criteria as specified in the test method cited above were satisfied.
- No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.
- Inflated swim bladders were confirmed in all test organisms used in this test.

Work Order : 221257
Sample Number : 33961

Fathead Minnow Growth Inhibition (based on Biomass)



REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519 [Program on disk and printed User's Guide].

[b] Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

[c] Stephan, C. E. 1977. Methods for calculating an LC50. pp 65-84 in : P. L. Mayer and J. L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation. Amer. Soc. Testing and Materials, Philadelphia PA. ASTM STP 634.

Date : 2012-05-09
yyyy-mm-dd

Approved By : [signature]
Project Manager

Work Order : 221257
Sample Number : 33961

CUMULATIVE DAILY CONTROL MORTALITY AND IMPAIRMENT (±SD)

Date :	2012-04-24	2012-04-25	2012-04-26	2012-04-27	2012-04-28	2012-04-29	2012-04-30	2012-05-01
	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)

FATHEAD MINNOW CUMULATIVE DAILY MORTALITY

Initiation Time : 14:00
Initiation Date : 2012-04-24
Completion Date : 2012-05-01

		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Treatment Mean Mortality (± SD)
Date :		2012-04-24		2012-04-25		2012-04-26		2012-04-27		2012-04-28		2012-04-29		2012-04-30		2012-05-01		
Analyst(s):		CN		XD		XD		VC		FS		FS		AW		JGG		
Concentration (%)	**Replicate**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	%
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
Control	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
1.56	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
3.13	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
6.25	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
12.5	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
25	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
50	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
100	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	

Aberrant behaviour or swimming impairment : None

Data Reviewed By: SM

Date : 2012-05-02

Work Order : 221257
Sample Number : 33961

FATHEAD MINNOW DRY WEIGHT AND BIOMASS DATA

Concentration (%)	Replicate	Number of Larvae Exposed	Replicate Mean Dry Weight (mg)	Treatment Mean Biomass (mg)	Standard Deviation
	A	10	0.918		
Control	B	10	1.021	0.961	0.054
	C	10	0.944		
	A	10	0.889		
1.56	B	10	0.884	0.873	0.024
	C	10	0.846		
	A	10	0.898		
3.13	B	10	0.898	0.916	0.031
	C	10	0.951		
	A	10	0.914		
6.25	B	10	0.947	0.916	0.030
	C	10	0.888		
	A	10	0.877		
12.5	B	10	0.913	0.863	0.058
	C	10	0.800		
	A	10	0.871		
25	B	10	0.999	0.891	0.100
	C	10	0.803		
	A	10	0.750		
50	B	10	0.813	0.794	0.039
	C	10	0.820		
	A	10	0.909		
100	B	10	0.842	0.838	0.073
	C	10	0.764		

NOTES :

- No outlying data points were detected according to Grubbs Test[b].
- Control average dry weight per surviving organism = 0.961 mg

Data Reviewed By: Sm

Date : 2012-05-02

Work Order : 221257
Sample Number: 33961

Fathead Minnow Water Chemistry Data

Initial Chemistry:	Temp. (°C)	DO (mg/L)	pH	Conductivity (μmhos/cm)	Hardness (mg/L as $CaCO_3$)
	25.0	8.4	8.5	502	250

		Day 0 - 1 2012-04-24	Day 1 - 2 2012-04-25	Day 2 - 3 2012-04-26	Day 3 - 4 2012-04-27	Day 4 - 5 2012-04-28	Day 5 - 6 2012-04-29	Day 6 - 7 2012-04-30
Sub-sample Used		1	1	1	2	2	3	3
Temperature (°C)		25.0	24.0	24.0	24.5	24.0	24.0	24.0
Dissolved Oxygen (mg/L)		8.4	8.0	8.9	8.7	8.9	8.8	8.8
Dissolved Oxygen % Sat.[1]		110	101	111	108	110	108	108
pH		8.5	8.4	8.4	8.5	8.4	8.4	8.4
Pre-aeration Time (min)[2]		20	20	20	20	20	20	20
Analyst(s) :	Initial	CL	CL	AW	AW	MR	CL	AW
	Final	CL	AW	CL	MR	CL	AW	HL(RD)
Control (0%)								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO % Sat.	Initial	95	98	97	97	97	100	97
DO (mg/L)	Initial	7.5	7.9	7.8	8.0	8.1	8.2	8.0
	Final	7.4	6.8	6.1	6.6	6.3	6.0	6.2
pH	Initial	8.3	8.3	8.3	8.4	8.4	8.3	8.3
	Final	8.2	8.0	7.9	8.1	7.9	7.8	7.7
Cond. (μmhos)	Initial	502	502	485	493	505	476	475
1.56 %								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.6	7.9	7.8	8.0	8.1	8.2	8.1
	Final	7.1	6.4	6.1	7.1	6.4	6.3	7.2
pH	Initial	8.4	8.3	8.3	8.4	8.4	8.4	8.0
	Final	8.2	7.9	7.9	8.1	7.9	7.8	7.9
Cond. (μmhos)	Initial	503	504	486	483	484	479	477
25 %								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.7	7.9	7.9	8.0	8.2	8.3	8.1
	Final	7.1	6.4	6.2	7.0	6.3	6.4	6.5
pH	Initial	8.4	8.4	8.4	8.4	8.4	8.4	8.4
	Final	8.3	8.0	8.0	8.2	8.0	8.0	7.9
Cond. (μmhos)	Initial	506	505	491	489	492	486	485
100 %								
Temp.(°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	8.2	8.0	8.4	8.4	8.3	8.5	8.3
	Final	7.0	6.4	5.9	6.8	6.7	6.1	5.9
pH	Initial	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	Final	8.5	8.3	8.3	8.4	8.4	8.3	8.2
Cond. (μmhos)	Initial	507	507	505	506	505	506	504

"–" = not measured

[1] % saturation (adjusted for actual temperature and barometric pressure)

[2] ≤100 bubbles/minute

AQUATOX

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Larval Fathead Minnow Test Report

Survival and Growth

Work Order : 221257
Sample Number : 33962

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-25
Sample Description :	Cloudy, orange, odourless		

Test Method : Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/22 , 2nd ed. (February 2011).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth from Biomass)	>100%	-	-
LC50	>100%	-	-

The results reported relate only to the sample tested.

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	2012-04-25	Analyst(s) :	RD/MR/CL
Organism Batch :	Fm12-04	Test Duration :	7 days
IC25 Growth (from Biomass) :	0.99 g/L	LC50 :	1.09 g/L
95% Confidence Limits :	0.83 - 1.05 g/L	95% Confidence Limits :	0.87 - 1.36 g/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Statistical Method :	Probit (Stephan)[c]
Historical Mean IC25 :	0.97 g/L	Historical Mean LC50 :	1.12 g/L
Warning Limits (± 2SD) :	0.82 - 1.14 g/L	Warning Limits (± 2SD) :	1.00 - 1.25 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

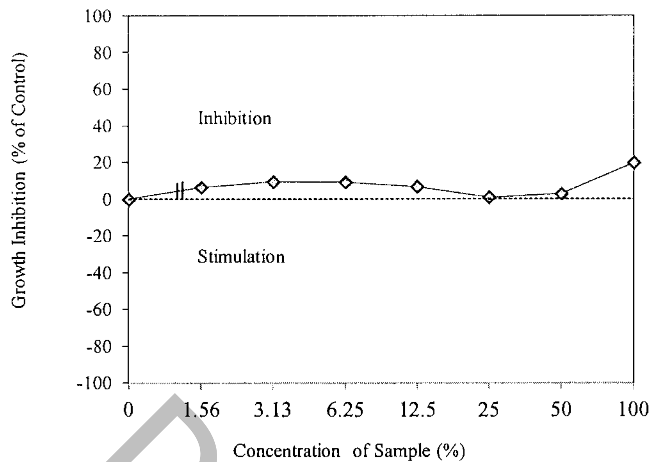
Test Organism :	*Pimephales promelas*	Test Type :	Static Renewal
Organism Batch :	Fm12-04	Control/Dilution Water :	Well water (no chemicals added)
Organism Age :	~07:00 - 22:05 h at start of test	Test Volume / Replicate :	300 mL
Source :	In-house culture	Test Vessel :	420 mL polystyrene beaker
Culture Mortality/Diseased :	0.07 % (previous 7 days)	Depth of Test Solution :	8 cm
pH Adjustment :	None	Organisms per Replicate :	10
Sample Filtration :	None	Number of Replicates :	3
Hardness Adjustment :	None	Daily Renewal Method :	80-85% syphoned and replaced
Test Aeration :	None	Test Method Deviation(s):	None

COMMENTS

- All test validity criteria as specified in the test method cited above were satisfied.
- No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.
- Inflated swim bladders were confirmed in all test organisms used in this test.

Work Order : 221257
Sample Number : 33962

Fathead Minnow Growth Inhibition (based on Biomass)



REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

[c] Stephan, C. E. 1977. Methods for calculating an LC50. pp 65-84 in : P. L. Mayer and J. L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation. Amer. Soc. Testing and Materials, Philadelphia PA. ASTM STP 634.

Date : ______________________ ***Approved By :*** ______________________

yyyy-mm-dd *Project Manager*

Work Order : 221257
Sample Number : 33962

CUMULATIVE DAILY CONTROL MORTALITY AND IMPAIRMENT (±SD)

Date :	2012-04-25	2012-04-26	2012-04-27	2012-04-28	2012-04-29	2012-04-30	2012-05-01	2012-05-02
	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)	0.00% (±0.0)

FATHEAD MINNOW CUMULATIVE DAILY MORTALITY

Initiation Time : 14:05
Initiation Date : 2012-04-25
Completion Date : 2012-05-02

		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Treatment Mean Mortality (± SD)
Date :		2012-04-25		2012-04-26		2012-04-27		2012-04-28		2012-04-29		2012-04-30		2012-05-01		2012-05-02		
Analyst(s):		VC		VC		CN		CL		FS		XD		AW		RD		
Concentration (%)	**Replicate**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	%
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
Control	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
1.56	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
3.13	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	**3.33 (±5.77)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
6.25	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
12.5	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
25	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
50	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
100	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00 (±0.00)**
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	

Aberrant behaviour or swimming impairment : None

Data Reviewed By: SM

Date : 2012-05-03

Work Order : 221257
Sample Number : 33962

FATHEAD MINNOW DRY WEIGHT AND BIOMASS DATA

Concentration (%)	Replicate	Number of Larvae Exposed	Replicate Mean Dry Weight (mg)	Treatment Mean Biomass (mg)	Standard Deviation
Control	A	10	0.976	1.004	0.032
	B	10	0.997		
	C	10	1.039		
1.56	A	10	0.933	0.939	0.045
	B	10	0.987		
	C	10	0.898		
3.13	A	10	0.893	0.908	0.062
	B	10	0.855		
	C	10	0.976		
6.25	A	10	0.885[1]	0.911	0.023
	B	10	0.924		
	C	10	0.925		
12.5	A	10	0.966	0.936	0.055
	B	10	0.969		
	C	10	0.872[1]		
25	A	10	0.996	0.994	0.044
	B	10	1.036		
	C	10	0.949		
50	A	10	0.858	0.974	0.199
	B	10	0.861		
	C	10	1.204		
100	A	10	0.674	0.805	0.117
	B	10	0.898		
	C	10	0.844		

NOTES :

- [1]Outlier according to Grubbs Test[b]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.
- Control average dry weight per surviving organism = 1.004 mg

Data Reviewed By: SM

Date : 2012-05-03

Work Order : 221257
Sample Number: 33962

Fathead Minnow Water Chemistry Data

Initial Chemistry:	Temp. (°C)	DO (mg/L)	pH	Conductivity (µmhos/cm)	Hardness (mg/L as $CaCO_3$)
	24.0	8.1	8.5	500	260

		Day 0 - 1 2012-04-25	Day 1 - 2 2012-04-26	Day 2 - 3 2012-04-27	Day 3 - 4 2012-04-28	Day 4 - 5 2012-04-29	Day 5 - 6 2012-04-30	Day 6 - 7 2012-05-01
Sub-sample Used		1	1	1	2	2	3	3
Temperature (°C)		24.0	24.0	24.5	24.0	24.0	24.0	24.5
Dissolved Oxygen (mg/L)		8.1	8.6	8.8	8.8	8.9	9.0	8.9
Dissolved Oxygen % Sat.[1]		102	108	110	108	109	110	112
pH		8.5	8.4	8.4	8.4	8.4	8.4	8.4
Pre-aeration Time (min)[2]		20	20	20	20	20	20	20
Analyst(s) :	Initial	CL	AW	AW	JGG	CL	AW	AW
	Final	CL	CL	JGG	CL	AW	HL(RD)	HL(RD)
Control (0%)								
Temp.(°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0	24.5
	Final	24.0	24.0	24.5	24.0	24.0	24.5	25.0
DO % Sat.	Initial	97	97	97	96	100	98	97
DO (mg/L)	Initial	7.8	7.7	7.9	8.0	8.2	8.0	7.7
	Final	7.5	6.3	7.2	6.7	6.6	6.2	5.6
pH	Initial	8.4	8.4	8.4	8.3	8.4	8.4	8.3
	Final	8.2	7.9	8.1	8.1	8.1	8.1	7.6
Cond. (µmhos)	Initial	500	484	482	478	476	477	474
1.56 %								
Temp.(°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0	24.5
	Final	24.0	24.0	24.5	24.0	24.0	24.5	25.0
DO (mg/L)	Initial	7.9	7.8	7.9	8.0	8.2	8.1	8.3
	Final	7.6	6.4	7.3	6.8	6.3	6.2	5.4
pH	Initial	8.3	8.4	8.4	8.4	8.4	8.3	8.3
	Final	8.2	7.9	8.1	8.0	7.9	8.0	7.7
Cond. (µmhos)	Initial	503	484	483	482	479	479	477
25 %								
Temp.(°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0	24.5
	Final	24.0	24.0	24.5	24.0	24.0	24.5	25.0
DO (mg/L)	Initial	7.9	7.9	8.0	8.0	8.3	8.1	8.0
	Final	7.3	6.4	7.1	6.5	6.2	7.3	5.2
pH	Initial	8.3	8.4	8.4	8.5	8.5	8.3	8.4
	Final	8.3	8.1	8.2	8.1	8.0	8.0	7.8
Cond. (µmhos)	Initial	502	491	489	489	487	490	485
100 %								
Temp.(°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0	24.5
	Final	24.0	24.0	24.5	24.0	24.0	24.5	25.0
DO (mg/L)	Initial	8.1	8.3	8.3	8.4	8.5	8.2	8.6
	Final	7.2	6.7	7.0	6.3	5.9	7.6	5.7
pH	Initial	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	Final	8.5	8.4	8.4	8.4	8.3	8.3	8.1
Cond. (µmhos)	Initial	506	504	505	506	506	507	506

"–" = not measured

[1] % saturation (adjusted for actual temperature and barometric pressure)

[2] ≤100 bubbles/minute

May 10, 2012

Chronic Bioassay Test Results:

Ceriodaphnia (crustacean)

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Ceriodaphnia dubia Test Report

Survival and Reproduction

Work Order : 221257
Sample Number : 33960

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process Water-2	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-24
Sample Description :	Clear, colourless, odourless.		

Test Method : Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia*. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21, 2nd ed. (February 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
LC50	>100%	-	-
IC25 (Reproduction)	63.5%	56.9-68.0	Linear Interpolation (CETIS) a

The results reported relate only to the sample tested.

SODIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	2012-04-25	Analyst(s) :	VC/MR/RD
Organism Batch :	Cd12-04	Test Duration :	7 days
IC25 Reproduction :	0.86 g/L	LC50 :	2.09 g/L
95% Confidence Limits :	0.76 - 1.11 g/L	95% Confidence Limits :	0.65 - 3.00 g/L
Statistical Method :	Linear Interpolation (CETIS)[a]	Statistical Method :	Nonlinear Interpolation (Stephan)[c]
Historical Mean IC25 :	1.04 g/L	Historical Mean LC50 :	2.17 g/L
Warning Limits (± 2SD) :	0.60 - 1.81 g/L	Warning Limits (± 2SD) :	1.45 - 3.24 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

Sample Filtration :	None	Test Volume per Replicate :	15 mL
Test Aeration :	None	Test Vessel :	22 mL polystyrene vial
pH Adjustment :	None	Depth of Test Solution :	4.0 cm
Hardness Adjustment :	None	Organisms per Replicate :	1
Daily Renewal Method :	Transferred to fresh solutions	Number of Replicates :	10
Control/Dilution Water :	Well water (no chemicals added)	Test Method Deviation(s) :	None

COMMENTS

•All test validity criteria as specified in the test method cited above were satisfied.
•Statistical analysis could not be performed using non linear regression, since a suitable model could not be found. Therefore, test results were calculated using Linear Interpolation (CETIS)[a].

Work Order : 221257
Sample Number : 33960

TEST ORGANISMS

Test Organism : *Ceriodaphnia dubia*
Organism Batch : Cd12-04
Organism Origin : Single in-house mass culture
Test Organism Origin : Individual in-house cultures

Range of Age (at start of test) : 06:00 h - 12:10 h
Mean Brood Organism Mortality : 3.3%
Ephippia in Culture : No

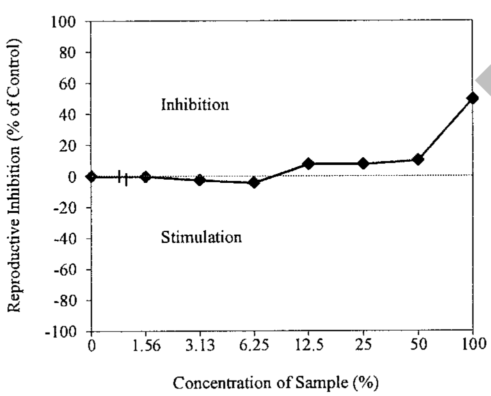
Brood Organism Neonate Production

Replicate :	1	2	3	4	5	6	7	8	9	10	Mean
Total (third or subsequent brood):	18	16	15	15	17	14	17	14	13	14	15.3
Total (first three broods):	27	24	21	26	28	23	25	27	27	25	25.3

No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.

TEST DATA

Ceriodaphnia dubia **Reproductive Inhibition**



Cumulative Daily Test Organism Mortality (%)

		Concentration of Sample (%)							
Date	**Test Day**	Control	1.56	3.13	6.25	12.5	25	50	100
2012-04-25	1	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0
2012-04-28	4	0	0	0	0	0	0	0	0
2012-04-29	5	0	0	0	0	0	0	0	0
2012-04-30	6	0	0	0	0	0	0	0	0
2012-05-01	7	0	0	0	0	0	0	0	0
Total Mortality (%)		0	0	0	0	0	0	0	0

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519[Program on disk and printed User's Guide].

[c] Stephan, C. E. 1977. Methods for calculating an LC50. pp 65-84 in : P. L. Mayer and J. L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation. Amer. Soc. Testing and Materials, Philadelphia PA. ASTM STP 634.

Date : ____________________
yyyy-mm-dd

Approved By : ____________________
Project Manager

Work Order : 221257
Sample Number : 33960

Ceriodaphnia dubia Survival and Reproduction

Test Initiation Date : 2012-04-24
Initiation Time : 15:30
Test Completion Date : 2012-05-01

Concentration (%) Control	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)	Analyst(s)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0	CL
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0	CL
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0	CL
2012-04-28	4	7	7	6	6	6	6	6	6	7	6	6.3	VC
2012-04-29	5	11	13	13	14	13	15	17	14	11	14	13.5	VC
2012-04-30	6	0	14	0	0	11	0	9	0	0	20	5.4	XD
2012-05-01	7	20	0	17	19	0	19	0	22	18	0	11.5	RD
Total		**38**	**34**	**36**	**39**	**30**	**40**	**32**	**42**	**36**	**40**	**36.7 (±3.8)**	

Concentration (%) 12.5	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	6	6	5	6	7	8	6	3	6	6	5.9
2012-04-29	5	13	11	13	13	15	13	11	6	13	12	12
2012-04-30	6	17	1	12	0	0	0	0	0	0	15	4.5
2012-05-01	7	0	17	0	17	21	18	15	11	16	0	11.5
Total		**36**	**35**	**30**	**36**	**43**	**39**	**32**	**20**	**35**	**33**	**33.9 (±6.1)**

Concentration (%) 1.56	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	6	5	7	8	6	7	8	8	4	6	6.5
2012-04-29	5	12	13	15	16	14	15	10	17	13	16	14.1
2012-04-30	6	4	0	18	0	0	0	0	0	0	19	4.1
2012-05-01	7	0	18	0	16	18	19	21	17	12	0	12.1
Total		**22**	**36**	**40**	**40**	**38**	**41**	**39**	**42**	**29**	**41**	**36.8 (±6.4)**

Concentration (%) 25	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	7	4	3	7	6	7	7	6	5	7	5.9
2012-04-29	5	12	10	15	12	12	12	15	13	14	14	12.9
2012-04-30	6	17	0	16	15	15	0	17	0	5	19	10.4
2012-05-01	7	0	14	0	0	0	18	0	15	0	0	4.7
Total		**36**	**28**	**34**	**34**	**33**	**37**	**39**	**34**	**24**	**40**	**33.9 (±4.8)**

Concentration (%) 3.13	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	6	6	7	6	7	8	7	6	5	6	6.4
2012-04-29	5	15	13	15	14	15	15	16	9	15	15	14.2
2012-04-30	6	14	0	7	0	0	0	0	0	0	26	4.7
2012-05-01	7	0	19	0	19	24	20	7	13	22	0	12.4
Total		**35**	**38**	**29**	**39**	**46**	**43**	**30**	**28**	**42**	**47**	**37.7 (±7.0)**

Concentration (%) 50	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	6	6	7	7	7	6	6	5	6	8	6.4
2012-04-29	5	11	11	13	14	12	11	12	9	11	11	11.5
2012-04-30	6	16	12	19	0	0	15	13	0	0	11	8.6
2012-05-01	7	0	0	0	17	15	0	0	14	19	0	6.5
Total		**33**	**29**	**39**	**38**	**34**	**32**	**31**	**28**	**36**	**30**	**33.0 (±3.7)**

Concentration (%) 6.25	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	8	6	7	6	6	5	9	3	6	8	6.4
2012-04-29	5	15	14	15	12	14	15	15	15	12	14	14.1
2012-04-30	6	19	18	0	0	0	0	0	0	18	16	7.1
2012-05-01	7	0	0	17	15	20	19	18	18	0	0	10.7
Total		**42**	**38**	**39**	**33**	**40**	**39**	**42**	**36**	**36**	**38**	**38.3 (±2.8)**

Concentration (%) 100	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	4	0	0	3	0	4	5	0	0	1	1.7
2012-04-29	5	6	0	4	0	0	6	9	7	0	4	3.6
2012-04-30	6	0	6	0	5	5	0	0	0	6	0	2.2
2012-05-01	7	11	10	10	11	9	11	10	13	10	15	11
Total		**21**	**16**	**14**	**19**	**14**	**21**	**24**	**20**	**16**	**20**	**18.5 (±3.3)**

NOTES : •All young produced by a test organism during its fourth and subsequent broods were discarded and not included in the above counts. The presence of two or more neonates in any test chamber, during any given day of the test, constitutes a brood.
•No outlying data points were detected according to Grubbs Test (CETIS)[a].

"x"= test organism mortality
"*"= accidental test organism mortality
"–"=4th brood (see 'NOTES')

Data Reviewed By : ________

Date : 2012-05-03

Work Order : 221257
Sample Number: 33960

Ceriodaphnia dubia Water Chemistry Data

Initial Chemistry:	Temp. (°C)	DO (mg/L)	pH	Conductivity (µmhos/cm)	Hardness (mg/L as $CaCO_3$)
	25.0	7.7	7.6	421	90

		Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 4	Day 4 - 5	Day 5 - 6	Day 6 - 7
Date :		**2012-04-24**	**2012-04-25**	**2012-04-26**	**2012-04-27**	**2012-04-28**	**2012-04-29**	**2012-04-30**
Sub-sample Used		1	1	1	2	2	3	3
Temperature (°C)		25.0	24.0	24.0	24.0	24.0	24.0	24.0
Dissolved Oxygen (mg/L)		7.7	7.2	8.2	7.7	8.1	8.5	8.7
Dissolved Oxygen % Sat.[3]		100	91	103	95	99	104	108
pH		7.6	7.6	7.6	7.6	7.6	7.7	7.7
Pre-aeration Time (min)[4]		0	0	20	0	0	20	20
Analyst(s)	Initial	CL	CL	AW	AW	JGG	CL	AW
	Final	CL	CL	CL	MR	CL	AW	HL(RD)
Control (0%)								
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO % Sat.[3]	Initial	95	89	98	95	97	100	97
DO (mg/L)	Initial	7.6	7.2	7.8	7.8	8.0	8.2	8.1
	Final	7.2	7.0	7.2	7.3	7.4	7.5	7.2
pH	Initial	8.3	8.3	8.4	8.2	8.3	8.4	8.3
	Final	8.1	8.0	8.1	8.1	8.2	8.3	7.8
Cond. (µmhos)	Initial	501	499	555	483	481	476	479
1.56 %								
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.6	7.8	7.9	8.0	8.3	8.2	8.1
	Final	7.0	7.1	7.1	7.3	7.6	7.5	7.1
pH	Initial	8.4	8.3	8.4	8.3	8.4	8.4	8.3
	Final	8.1	8.0	8.1	8.2	8.2	8.2	8.0
Cond. (µmhos)	Initial	502	501	485	482	481	477	475
25 %								
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.7	7.8	7.9	8.1	8.0	8.3	8.2
	Final	7.1	7.1	7.3	7.3	7.6	7.6	7.3
pH	Initial	8.2	8.2	8.3	8.2	8.3	8.3	8.3
	Final	8.1	8.1	8.1	8.2	8.2	8.2	8.0
Cond. (µmhos)	Initial	485	484	472	471	454	462	460
100 %								
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.6	7.7	8.0	7.9	8.3	8.4	8.4
	Final	7.2	7.2	7.3	7.4	7.4	7.5	7.3
pH	Initial	7.8	7.9	8.0	7.9	8.0	8.1	8.0
	Final	8.2	8.1	8.2	8.2	8.2	8.2	8.1
Cond. (µmhos)	Initial	425	426	423	427	429	405	405

"–" = not measured

[3] % saturation (adjusted for actual temperature and barometric pressure)

[4] $\leq$100 bubbles/minute

AQUATOX

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33961

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process + Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-24
Sample Description :	Clear, orange, odourless		

Test Method : Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia*. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21, 2nd ed. (February 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
LC50	>100%	-	-
IC25 (Reproduction)	74.8%	65.2-84.7	Linear Interpolation (CETIS) a

The results reported relate only to the sample tested.

SODIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	2012-04-25	Analyst(s) :	VC/MR/RD
Organism Batch :	Cd12-04	Test Duration :	7 days
IC25 Reproduction :	0.86 g/L	LC50 :	2.09 g/L
95% Confidence Limits :	0.76 - 1.11 g/L	95% Confidence Limits :	0.65 - 3.00 g/L
Statistical Method :	Linear Interpolation (CETIS)[a]	Statistical Method :	Nonlinear Interpolation (Stephan)[c]
Historical Mean IC25 :	1.04 g/L	Historical Mean LC50 :	2.17 g/L
Warning Limits (± 2SD) :	0.60 - 1.81 g/L	Warning Limits (± 2SD) :	1.45 - 3.24 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

Sample Filtration :	None	Test Volume per Replicate :	15 mL
Test Aeration :	None	Test Vessel :	22 mL polystyrene vial
pH Adjustment :	None	Depth of Test Solution :	4.0 cm
Hardness Adjustment :	None	Organisms per Replicate :	1
Daily Renewal Method :	Transferred to fresh solutions	Number of Replicates :	10
Control/Dilution Water :	Well water (no chemicals added)	Test Method Deviation(s) :	None

COMMENTS

•All test validity criteria as specified in the test method cited above were satisfied.
•Statistical analysis could not be performed using non linear regression, since a suitable model could not be found. Therefore, test results were calculated using Linear Interpolation (CETIS)[a].

Work Order : 221257
Sample Number : 33961

TEST ORGANISMS

Test Organism :	*Ceriodaphnia dubia*	Range of Age (at start of test) :	12:15 h - 22:50 h
Organism Batch :	Cd12-04	Mean Brood Organism Mortality :	3.3%
Organism Origin :	Single in-house mass culture	Ephippia in Culture :	No
Test Organism Origin :	Individual in-house cultures		

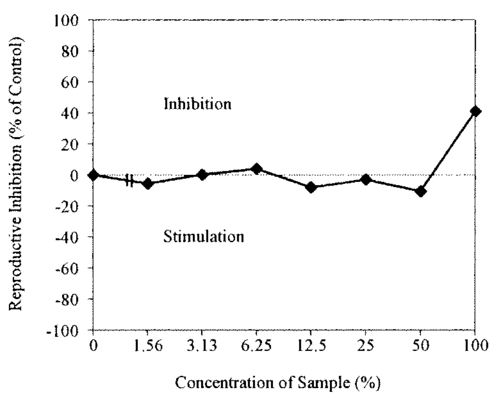
Brood Organism Neonate Production

Replicate :	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
Total (third or subsequent brood):	18	15	17	17	20	16	18	17	19	17	17.4
Total (first three broods):	25	24	26	28	27	25	22	26	26	19	24.8

No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.

TEST DATA

***Ceriodaphnia dubia* Reproductive Inhibition**



Cumulative Daily Test Organism Mortality (%)

		Concentration of Sample (%)							
Date	**Test Day**	Control	1.56	3.13	6.25	12.5	25	50	100
2012-04-25	1	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0
2012-04-28	4	0	0	0	0	0	0	0	0
2012-04-29	5	0	0	10	10	0	10	0	0
2012-04-30	6	0	0	10	10	0	10	0	0
Total Mortality (%)		0	0	10	10	0	10	0	0

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519[Program on disk and printed User's Guide].

[c] Stephan, C. E. 1977. Methods for calculating an LC50. pp 65-84 in : P. L. Mayer and J. L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation. Amer. Soc. Testing and Materials, Philadelphia PA. ASTM STP 634.

Date : 2012-05-09
yyyy-mm-dd

Approved By : [signature]
Project Manager

AQUATOX

Work Order : 221257
Sample Number : 33961

Ceriodaphnia dubia Survival and Reproduction

Test Initiation Date : 2012-04-24
Initiation Time : 15:35
Test Completion Date : 2012-04-30

Concentration (%) Control	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)	Analyst(s)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0	XD
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0	XD
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0	RD
2012-04-28	4	7	6	6	6	6	6	6	6	5	6	6.0	MR
2012-04-29	5	11	13	12	9	12	15	11	12	12	14	12.1	CL
2012-04-30	6	21	19	14	22	17	18	11	0	16	25	16.3	XD
Total		**39**	**38**	**32**	**37**	**35**	**39**	**28**	**18**	**33**	**45**	**34.4 (±7.4)**	

Concentration (%) 12.5	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	7	6	6	6	5	6	6	7	6	6	6.1
2012-04-29	5	15	13	12	12	8	12	12	14	11	14	12.3
2012-04-30	6	21	17	19	20	17	19	20	19	18	17	18.7
Total		**43**	**36**	**37**	**38**	**30**	**37**	**38**	**40**	**35**	**37**	**37.1 (±3.3)**

Concentration (%) 1.56	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	7	7	6	7	6	6	5	6	7	6	6.3
2012-04-29	5	11	12	10	13	11	11	10	8	12	13	11.1
2012-04-30	6	23	22	21	18	18	23	21	14	17	12	18.9
Total		**41**	**41**	**37**	**38**	**35**	**40**	**36**	**28**	**36**	**31**	**36.3 (±4.2)**

Concentration (%) 25	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	7	6	6	6	7	6	6	5	5	6	6.0
2012-04-29	5	14 x	13	11	10	12	12	15	11	10	12	12.0
2012-04-30	6	0	21	17	18	19	19	22	19	19	20	17.4
Total		**21**	**40**	**34**	**34**	**38**	**37**	**43**	**35**	**34**	**38**	**35.4 (±5.9)**

Concentration (%) 3.13	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	7	6	6	5	6	7	6	4	6	6	5.9
2012-04-29	5	15	13	12	12	13	14	12	11	0 x	12	11.4
2012-04-30	6	21	20	20	19	18	15	17	19	0	21	17.0
Total		**43**	**39**	**38**	**36**	**37**	**36**	**35**	**34**	**6**[1]	**39**	**34.3 (±10.3)**

Concentration (%) 50	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	7	7	6	7	6	7	6	6	5	4	6.1
2012-04-29	5	14	11	14	14	12	14	12	13	10	14	12.8
2012-04-30	6	21	20	20	17	23	18	19	13	19	21	19.1
Total		**42**	**38**	**40**	**38**	**41**	**39**	**37**	**32**	**34**	**39**	**38.0 (±3.1)**

Concentration (%) 6.25	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	6	6	4	6	7	7	6	6	6	4	5.8
2012-04-29	5	15	8	1 x	12	13	13	11	12	11	15	11.1
2012-04-30	6	17	19	0	19	20	18	15	19	12	22	16.1
Total		**38**	**33**	**5**	**37**	**40**	**38**	**32**	**37**	**29**	**41**	**33.0 (±10.5)**

Concentration (%) 100	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-25	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-26	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	3	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	4	6	5	4	4	7	6	5	4	5	5	5.1
2012-04-29	5	10	13	9	10	10	9	10	10	11	10	10.2
2012-04-30	6	7	14	1	0	0	0	0	11	16	0	4.9
Total		**23**	**32**	**14**	**14**	**17**	**15**	**15**	**25**	**32**	**15**	**20.2 (±7.3)**

NOTES : •All young produced by a test organism during its fourth and subsequent broods were discarded and not included in the above counts. The presence of two or more neonates in any test chamber, during any given day of the test, constitutes a brood.
•[1] Outlier according to Grubbs Test (CETIS)[a]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

"x"= test organism mortality
"*"= accidental test organism mortality
"–"=4th brood (see 'NOTES')

Data Reviewed By : SM
Date : 2012-05-02

Work Order : 221257
Sample Number: 33961

Ceriodaphnia dubia Water Chemistry Data

Initial Chemistry:	Temp. (°C)	DO (mg/L)	pH	Conductivity (µmhos/cm)	Hardness (mg/L as $CaCO_3$)
	25.0	8.4	8.5	502	250

		Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 4	Day 4 - 5	Day 5 - 6
Date :		**2012-04-24**	**2012-04-25**	**2012-04-26**	**2012-04-27**	**2012-04-28**	**2012-04-29**
Sub-sample Used		1	1	1	2	2	3
Temperature (°C)		25.0	24.0	24.0	24.5	24.0	24.0
Dissolved Oxygen (mg/L)		8.4	8.0	8.9	8.7	8.9	8.8
Dissolved Oxygen % Sat.[3]		110	101	111	108	110	108
pH		8.5	8.4	8.4	8.5	8.4	8.4
Pre-aeration Time (min)[4]		20	20	20	20	20	20
Analyst(s)	Initial	CL	CL	AW	AW	MR	CL
	Final	CL	CL	CL	MR	CL	AW
Control (0%)							
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0
DO % Sat.[3]	Initial	95	98	97	97	97	100
DO (mg/L)	Initial	7.5	7.9	7.8	8.0	8.1	8.2
	Final	7.2	7.1	7.4	7.5	7.1	7.8
pH	Initial	8.3	8.3	8.3	8.4	8.4	8.3
	Final	8.1	8.1	8.1	8.3	8.2	8.2
Cond. (µmhos)	Initial	502	502	485	493	505	476
1.56 %							
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0
DO (mg/L)	Initial	7.6	7.9	7.8	8.0	8.1	8.2
	Final	7.2	7.1	7.3	7.4	7.1	7.3
pH	Initial	8.4	8.3	8.3	8.4	8.4	8.4
	Final	8.1	8.1	8.1	8.2	8.1	8.1
Cond. (µmhos)	Initial	503	504	486	483	484	479
25 %							
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0
DO (mg/L)	Initial	7.7	7.9	7.9	8.0	8.2	8.3
	Final	7.2	7.1	7.1	7.4	7.1	7.3
pH	Initial	8.4	8.4	8.4	8.4	8.4	8.4
	Final	8.2	8.2	8.2	8.3	8.2	8.2
Cond. (µmhos)	Initial	506	505	491	489	492	486
100 %							
Temp. (°C)	Initial	24.0	24.0	24.0	24.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0
DO (mg/L)	Initial	8.2	8.0	8.4	8.4	8.3	8.5
	Final	6.9	6.8	7.0	7.2	6.7	7.0
pH	Initial	8.5	8.5	8.5	8.5	8.5	8.5
	Final	8.4	8.4	8.4	8.3	8.4	8.4
Cond. (µmhos)	Initial	507	507	505	506	505	506

"--" = not measured

[3] % saturation (adjusted for actual temperature and barometric pressure)

[4] ≤100 bubbles/minute

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33962

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-25
Sample Description :	Cloudy, orange, odourless		

Test Method : Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia*. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21, 2nd ed. (February 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
LC50	>100%	-	-
IC25 (Reproduction)	>100%	-	-

The results reported relate only to the sample tested.

SODIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	2012-04-25	Analyst(s) :	VC/MR/RD
Organism Batch :	Cd12-04	Test Duration :	7 days
IC25 Reproduction :	0.86 g/L	LC50 :	2.09 g/L
95% Confidence Limits :	0.76 - 1.11 g/L	95% Confidence Limits :	0.65 - 3.00 g/L
Statistical Method :	Linear Interpolation (CETIS)[a]	Statistical Method :	Nonlinear Interpolation (Stephan)[c]
Historical Mean IC25 :	1.04 g/L	Historical Mean LC50 :	2.17 g/L
Warning Limits (± 2SD) :	0.60 - 1.81 g/L	Warning Limits (± 2SD) :	1.45 - 3.24 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

Sample Filtration :	None	Test Volume per Replicate :	15 mL
Test Aeration :	None	Test Vessel :	22 mL polystyrene vial
pH Adjustment :	None	Depth of Test Solution :	4.0 cm
Hardness Adjustment :	None	Organisms per Replicate :	1
Daily Renewal Method :	Transferred to fresh solutions	Number of Replicates :	10
Control/Dilution Water :	Well water (no chemicals added)	Test Method Deviation(s) :	None

COMMENTS

- All test validity criteria as specified in the test method cited above were satisfied.

Work Order : 221257
Sample Number : 33962

TEST ORGANISMS

Test Organism :	*Ceriodaphnia dubia*	Range of Age (at start of test) :	19:30 h - 20:40 h
Organism Batch :	Cd12-04	Mean Brood Organism Mortality :	0%
Organism Origin :	Single in-house mass culture	Ephippia in Culture :	No
Test Organism Origin :	Individual in-house cultures		

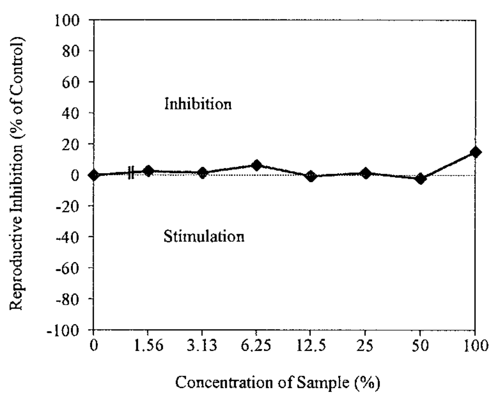
Brood Organism Neonate Production

Replicate :	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
Total (third or subsequent brood):	19	18	20	20	20	18	22	23	16	18	19.4
Total (first three broods):	28	31	31	31	30	33	29	32	27	30	30.2

No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.

TEST DATA

Ceriodaphnia dubia **Reproductive Inhibition**



Cumulative Daily Test Organism Mortality (%)

		Concentration of Sample (%)							
Date	**Test Day**	Control	1.56	3.13	6.25	12.5	25	50	100
2012-04-26	1	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0
2012-04-28	3	0	0	0	0	0	0	0	0
2012-04-29	4	0	0	0	0	0	0	0	0
2012-04-30	5	0	0	0	0	0	10	0	0
2012-05-01	6	0	0	0	0	0	10	0	0
Total Mortality (%)		0	0	0	0	0	10	0	0

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519[Program on disk and printed User's Guide].

[b] Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11*:1-21.

[c] Stephan, C. E. 1977. Methods for calculating an LC50. pp 65-84 in : P. L. Mayer and J. L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation. Amer. Soc. Testing and Materials, Philadelphia PA. ASTM STP 634.

Date : 2012-05-09
yyyy-mm-dd

Approved By : [signature]
Project Manager

AQUATOX

Work Order : 221257
Sample Number : 33962

Ceriodaphnia dubia Survival and Reproduction

Test Initiation Date : 2012-04-25
Initiation Time : 11:00
Test Completion Date : 2012-05-01

Concentration (%) Control	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)	Analyst(s)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0	VC
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0	VC
2012-04-28	3	6	7	6	4	6	6	6	6	5	6	5.8	MR
2012-04-29	4	0	0	0	0	0	0	0	0	0	0	0	JGG
2012-04-30	5	11	13	13	11	12	11	12	11	11	10	11.5	AW
2012-05-01	6	18	18	17	16	17	17	17	17	17	15	16.9	AW
Total		**35**	**38**	**36**	**31**	**35**	**34**	**35**	**34**	**33**	**31**	**34.2 (±2.1)**	

Concentration (%) 12.5	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	3	7	6	6	5	4	5	6	6	6	7	5.8
2012-04-29	4	0	0	0	0	0	0	0	0	0	0	0
2012-04-30	5	12	14	12	9	10	12	10	10	14	13	11.6
2012-05-01	6	18	18	19	12	18	20	16	16	19	14	17.0
Total		**37**	**38**	**37**	**26**	**32**	**37**	**32**	**32**	**39**	**34**	**34.4 (±4.0)**

Concentration (%) 1.56	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	3	7	6	5	6	6	7	6	3	5	5	5.6
2012-04-29	4	0	0	0	0	0	0	0	0	18	0	1.8
2012-04-30	5	12	12	12	12	11	13	11	0	0	14	9.7
2012-05-01	6	17	17	17	18	17	17	15	10	16	18	16.2
Total		**36**	**35**	**34**	**36**	**34**	**37**	**32**	**13**[1]	**39**	**37**	**33.3 (±7.4)**

Concentration (%) 25	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	3	5	7	6	4	6	8	6	6	7	6	6.1
2012-04-29	4	0	0	0	0	0	0	0	0	0	0	0
2012-04-30	5	11	13	10	12	12	13	14	12 x	11	14	12.2
2012-05-01	6	17	16	20	16	18	19	15	0	16	17	15.4
Total		**33**	**36**	**36**	**32**	**36**	**40**	**35**	**18**[1]	**34**	**37**	**33.7 (±5.9)**

Concentration (%) 3.13	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	3	6	7	7	5	6	5	5	6	3	5	5.5
2012-04-29	4	0	0	0	0	0	0	0	0	0	0	0
2012-04-30	5	12	11	13	12	9	13	13	12	12	10	11.7
2012-05-01	6	16	18	18	19	17	17	14	16	15	15	16.5
Total		**34**	**36**	**38**	**36**	**32**	**35**	**32**	**34**	**30**	**30**	**33.7 (±2.7)**

Concentration (%) 50	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	3	7	6	7	6	4	5	6	6	6	6	5.9
2012-04-29	4	0	0	0	0	0	0	0	0	0	0	0
2012-04-30	5	13	10	10	11	11	12	12	12	13	10	11.4
2012-05-01	6	16	17	16	18	20	18	17	21	15	18	17.6
Total		**36**	**33**	**33**	**35**	**35**	**35**	**35**	**39**	**34**	**34**	**34.9 (±1.7)**

Concentration (%) 6.25	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	3	4	5	6	6	6	2	6	4	5	7	5.1
2012-04-29	4	0	0	0	0	0	0	0	0	0	0	0
2012-04-30	5	12	12	12	12	11	8	11	11	0	11	10.0
2012-05-01	6	19	17	15	16	18	13	18	17	17	19	16.9
Total		**35**	**34**	**33**	**34**	**35**	**23**	**35**	**32**	**22**	**37**	**32.0 (±5.2)**

Concentration (%) 100	Day	1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2012-04-26	1	0	0	0	0	0	0	0	0	0	0	0
2012-04-27	2	0	0	0	0	0	0	0	0	0	0	0
2012-04-28	3	6	5	3	4	5	5	5	4	4	6	4.7
2012-04-29	4	0	0	0	0	0	0	0	0	0	0	0
2012-04-30	5	6	10	8	9	10	10	7	8	8	11	8.7
2012-05-01	6	16	18	16	15	16	17	10	14	17	17	15.6
Total		**28**	**33**	**27**	**28**	**31**	**32**	**22**	**26**	**29**	**34**	**29.0 (±3.6)**

NOTES : •All young produced by a test organism during its fourth and subsequent broods were discarded and not included in the above counts. The presence of two or more neonates in any test chamber, during any given day of the test, constitutes a brood.
•[1] Outlier according to Grubbs Test[b]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

"x"= test organism mortality
"*"= accidental test organism mortality
"–"=4th brood (see 'NOTES')

Data Reviewed By : [illegible]
Date : 2012-05-03

Work Order : 221257
Sample Number: 33962

Ceriodaphnia dubia Water Chemistry Data

Initial Chemistry:	Temp. (°C)	DO (mg/L)	pH	Conductivity (μmhos/cm)	Hardness (mg/L as $CaCO_3$)
	24.0	8.1	8.5	500	260

		Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 4	Day 4 - 5	Day 5 - 6
Date :		**2012-04-25**	**2012-04-26**	**2012-04-27**	**2012-04-28**	**2012-04-29**	**2012-04-30**
Sub-sample Used		1	1	1	2	2	3
Temperature (°C)		24.0	24.0	24.5	24.0	24.0	24.0
Dissolved Oxygen (mg/L)		8.1	8.6	8.8	8.8	8.9	9.0
Dissolved Oxygen % Sat.[3]		102	108	110	108	109	110
pH		8.5	8.4	8.4	8.4	8.4	8.4
Pre-aeration Time (min)[4]		20	20	20	20	20	20
Analyst(s)	Initial	CL	AW	AW	JGG	CL	AW
	Final	CL	AW	JGG	CL	AW	HL(RD)
Control (0%)							
Temp. (°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.5
DO % Sat.[3]	Initial	97	97	97	96	100	98
DO (mg/L)	Initial	7.8	7.7	7.9	8.0	8.2	8.0
	Final	7.2	7.3	7.4	7.5	7.4	7.4
pH	Initial	8.4	8.4	8.4	8.3	8.4	8.4
	Final	8.1	8.1	8.1	8.2	8.2	7.4
Cond. (μmhos)	Initial	500	484	482	478	476	477
1.56 %							
Temp. (°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.9	7.8	7.9	8.0	8.2	8.1
	Final	7.3	7.3	7.4	7.6	7.5	7.3
pH	Initial	8.3	8.4	8.4	8.4	8.4	8.3
	Final	8.2	8.1	8.2	8.3	8.1	8.0
Cond. (μmhos)	Initial	503	484	483	482	479	479
25 %							
Temp. (°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	7.9	7.9	8.0	8.0	8.3	8.1
	Final	7.2	7.2	7.3	7.4	7.4	7.4
pH	Initial	8.3	8.4	8.4	8.5	8.5	8.3
	Final	8.2	8.2	8.3	8.3	8.2	8.1
Cond. (μmhos)	Initial	502	491	489	489	487	490
100 %							
Temp. (°C)	Initial	24.0	24.0	24.0	25.0	24.0	24.0
	Final	24.0	24.0	24.0	24.0	24.0	24.5
DO (mg/L)	Initial	8.1	8.3	8.3	8.4	8.5	8.2
	Final	7.0	7.0	7.2	7.4	7.3	6.9
pH	Initial	8.5	8.5	8.5	8.5	8.5	8.5
	Final	8.4	8.4	8.5	8.5	8.4	8.3
Cond. (μmhos)	Initial	506	504	505	506	506	507

"–" = not measured

[3] % saturation (adjusted for actual temperature and barometric pressure)

[4] ≤100 bubbles/minute

AVALON RARE METALS INC.

May 10, 2012

Chronic Bioassay Test Results:
Lemna Minor (duck weed)

AQUATOX

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33960

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process Water-2	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-26
Sample Description :	Clear, colourless, odourless.		

Test Method : Test for Measuring the Inhibition of Growth using the Freshwater Macrophyte, *Lemna minor*. Method Development and Application Section, Environmental Technology Centre, Environment Canada. Ottawa, Ontario. Report EPS 1/RM/37, 2nd ed. (January 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Weight)	>97.0%	-	-
IC25 (Frond Production)	>97.0%	-	-

The results reported relate only to the sample tested.

DRAFT

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	Statistical Method :
Organism Batch :	Historical Geometric Mean IC25 :
Test Duration :	Warning Limits (± 2SD) :
IC25 (Frond Production) :	Growth Medium :
95% Confidence Limits :	Analyst(s) :

The reference toxicant test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

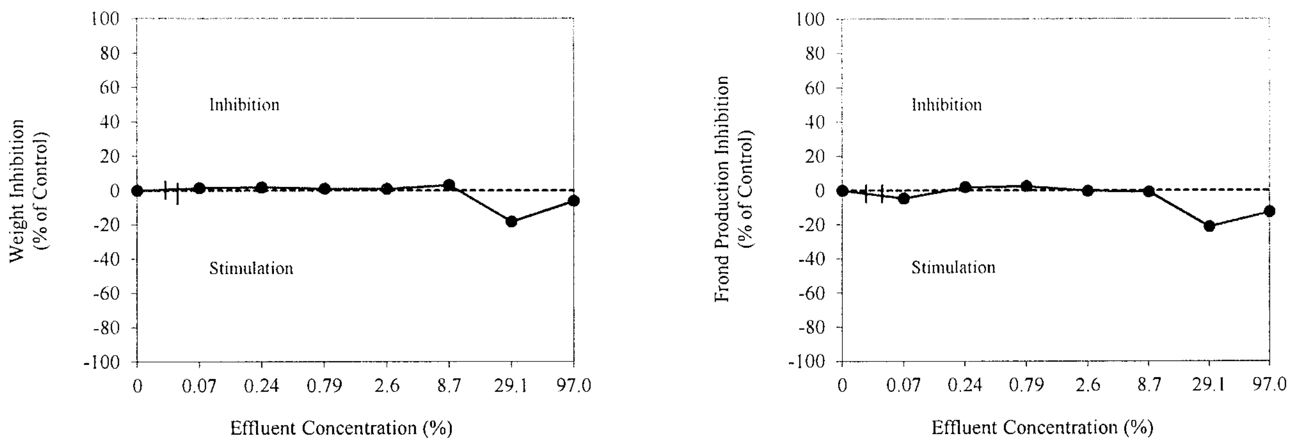
Test Organism :	*Lemna minor* L., Strain 7730	Test Type :	Static (no sub-samples required)
Organism Batch :	Lm12-04	Control/Dilution Medium :	Modified APHA
Culture Origin :	UTCC 492	Medium Preparation Water :	Distilled Water
Test Organism Source :	Axenic in-house culture	Source of Water :	Fernbrook Springs
Culture Medium :	Modified Hoaglands E+	Medium Preparation Chemicals :	Modified APHA stocks A, B, C (10 mL/L)
Age (on Test Day 0) :	9 days	Nutrient Spiking of Sample :	Modified APHA stocks A, B, C (10 mL/L)
Health Criteria (in APHA) :	15.7-fold frond increase in 7 days	Replicates per Concentration :	4
Organism Acclimation :	20:50 h in APHA medium	Test Volume per Replicate :	100 mL
Inoculum (Test Day 0) :	2 plants (3 fronds per plant)	Test Vessel :	250 mL glass Erlenmeyer flask
Sample Filtration :	1 µm (Whatman GF/C)	Depth of Test Solution :	4.0 cm
Sample Pre-aeration :	20 min. at ≤100 bubbles/min.	Photoperiod/Light Intensity :	Continuous, 4313 - 5005 lux
pH Adjustment :	None	Test Method Deviation(s) :	None
Hardness Adjustment :	None		

COMMENTS

•All test validity criteria as specified in the test method cited above were satisfied.

Work Order : 221257
Sample Number : 33960

Lemna minor Growth Inhibition



TEST MONITORING

Initiation Date :	2012-04-26	Termination Date :	2012-05-03
Intitiation Time :	12:05	Termination Time :	15:00
Initiated By :	SM	Terminated By :	AW

Temperature Monitoring

Test Day	**Date**	**Temperature (°C)**
0 (unmodified sample)	2012-04-26	25.0
0	2012-04-26	26.0
1	2012-04-27	26.0
2	2012-04-28	26.5
3	2012-04-29	25.5
4	2012-04-30	26.0
5	2012-05-01	26.0
6	2012-05-02	26.0
7	2012-05-03	25.5

pH Monitoring

Concentration (%)	**Day 0**	**Day 7**
100 (unmodified sample)	7.3	–
Control	8.2	8.3
0.07	8.2	8.3
0.24	–	–
0.79	–	–
2.6	8.2	8.3
8.7	–	–
29.1	–	–
97.0	7.9	8.7

"–" = not required

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519 [Program on disk and printed User's Guide].

Date : ______________________ *yyyy-mm-dd*

Approved By: ______________________ *Project Manager*

Work Order : 221257
Sample Number : 33960

Lemna minor Frond Increase

Concentration (%)	Replicate	Frond Count Day 0*	Frond Count Day 7	Frond Increase	Mean Frond Increase	Standard Deviation	CV (%)	Stimulation (%)**	Frond/Root Appearance (Day 7)
Control	A	6	88	82	90.75	8.22	9.1	–	Fronds healthy, appearance normal in all replicates.
	B	6	106	100					
	C	6	92	86					
	D	6	101	95					
0.07	A	6	99	93	95.00	3.37	3.5	–	Fronds healthy, appearance normal in all replicates.
	B	6	100	94					
	C	6	99	93					
	D	6	106	100					
0.24	A	6	83	77	89.00	10.80	12.1	–	Fronds healthy, appearance normal in all replicates.
	B	6	92	86					
	C	6	96	90					
	D	6	109	103					
0.79	A	6	99	93	88.50	4.65	5.3	–	Fronds healthy, appearance normal in all replicates.
	B	6	96	90					
	C	6	95	89					
	D	6	88	82					
2.6	A	6	99	93	91.00	4.90	5.4	–	Fronds healthy, appearance normal in all replicates.
	B	6	93	87					
	C	6	93	87					
	D	6	103	97					
8.7	A	6	105	99	91.50	8.70	9.5	–	Fronds healthy, appearance normal in all replicates.
	B	6	101	95					
	C	6	99	93					
	D	6	85	79					
29.1	A	6	94	88	110.00	16.08	14.6	21.2	Fronds healthy, appearance normal in all replicates.
	B	6	132	126					
	C	6	122	116					
	D	6	116	110					
97.0	A	6	108	102	102.25	8.73	8.5	–	Fronds healthy, appearance normal in all replicates.
	B	6	96	90					
	C	6	114	108					
	D	6	115	109					

NOTES: *No unusual appearance or treatment of culture prior to testing. Test inoculated with healthy plants.
**Significant stimulation (ANOVA - Dunnett's Test (CETIS)a, α=0.05) compared to control.
•A 16.1-fold increase in frond number was observed in the control over the testing period.
•No outlying data points were detected according to Grubbs Test (CETIS)[a].

"–" = not available/not required

Test Data Reviewed By : ______
Date : 2012-05-09

Work Order : 221257
Sample Number : 33960

Lemna minor Frond Weight Data

Concentration (%)	Replicate	Dry Weight of Fronds (mg)	Treatment Mean Dry Weight (mg)	Standard Deviation	Stimulation (%)**
Control	A	9.29	9.85	0.73	–
	B	10.59			
	C	9.17			
	D	10.36			
0.07	A	9.78	9.71	0.22	–
	B	9.99			
	C	9.52			
	D	9.56			
0.24	A	7.98	9.67	1.30	–
	B	9.53			
	C	10.04			
	D	11.11			
0.79	A	10.97	9.75	0.89	–
	B	9.85			
	C	8.97			
	D	9.21			
2.6	A	9.98	9.76	0.46	–
	B	9.53			
	C	9.25			
	D	10.28			
8.7	A	10.35	9.55	0.74	–
	B	9.97			
	C	9.13			
	D	8.75			
29.1	A	9.64	11.65	1.70	18.27
	B	13.81			
	C	11.57			
	D	11.59			
97.0	A	10.62	10.46	0.73	–
	B	9.56			
	C	11.33			
	D	10.33			

NOTES : **Significant stimulation (ANOVA - Dunnett's Test (CETIS)a, α=0.05) compared to control.
•No outlying data points were detected according to Grubbs Test (CETIS)[a].

"–" = not available/not required

Test Data Reviewed By : ______
Date : 2012-05-07

AQUATOX

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33961

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process + Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-26
Sample Description :	Clear, orange, odourless		

Test Method : Test for Measuring the Inhibition of Growth using the Freshwater Macrophyte, *Lemna minor*. Method Development and Application Section, Environmental Technology Centre, Environment Canada. Ottawa, Ontario. Report EPS 1/RM/37, 2nd ed. (January 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Weight)	>97.0%	-	-
IC25 (Frond Production)	>97.0%	-	-

The results reported relate only to the sample tested.

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :		Statistical Method :	
Organism Batch :		Historical Geometric Mean IC25 :	
Test Duration :		Warning Limits (± 2SD) :	
IC25 (Frond Production) :		Growth Medium :	
95% Confidence Limits :		Analyst(s) :	

The reference toxicant test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

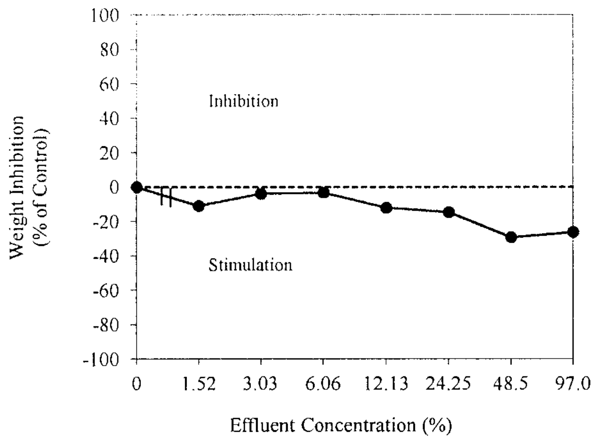
Test Organism :	*Lemna minor* L., Strain 7730	Test Type :	Static (no sub-samples required)
Organism Batch :	Lm12-04	Control/Dilution Medium :	Modified APHA
Culture Origin :	UTCC 492	Medium Preparation Water :	Distilled Water
Test Organism Source :	Axenic in-house culture	Source of Water :	Fernbrook Springs
Culture Medium :	Modified Hoaglands E+	Medium Preparation Chemicals :	Modified APHA stocks A, B, C (10 mL/L)
Age (on Test Day 0) :	9 days	Nutrient Spiking of Sample :	Modified APHA stocks A, B, C (10 mL/L)
Health Criteria (in APHA) :	15.7-fold frond increase in 7 days	Replicates per Concentration :	4
Organism Acclimation :	21:05 h in APHA medium	Test Volume per Replicate :	100 mL
Inoculum (Test Day 0) :	2 plants (3 fronds per plant)	Test Vessel :	250 mL glass Erlenmeyer flask
Sample Filtration :	1 µm (Whatman GF/C)	Depth of Test Solution :	4.0 cm
Sample Pre-aeration :	20 min. at ≤100 bubbles/min.	Photoperiod/Light Intensity :	Continuous, 4950 - 5442 lux
pH Adjustment :	None	Test Method Deviation(s) :	None
Hardness Adjustment :	None		

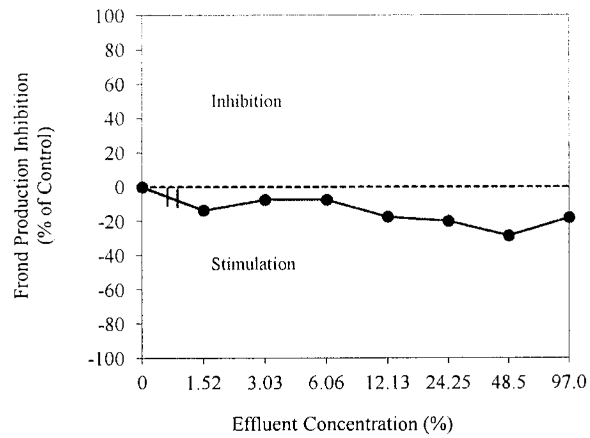
COMMENTS

•All test validity criteria as specified in the test method cited above were satisfied.

Work Order : 221257
Sample Number : 33961

Lemna minor Growth Inhibition





TEST MONITORING

DRAFT

Initiation Date :	2012-04-26	Termination Date :	2012-05-03
Intitiation Time :	12:20	Termination Time :	13:30
Initiated By :	AS	Terminated By :	AS

Temperature Monitoring

Test Day	Date	Temperature (°C)
0 (unmodified sample)	2012-04-26	25.0
0	2012-04-26	25.0
1	2012-04-27	25.0
2	2012-04-28	24.5
3	2012-04-29	24.5
4	2012-04-30	24.5
5	2012-05-01	24.5
6	2012-05-02	24.5
7	2012-05-03	24.5

pH Monitoring

Concentration (%)	Day 0	Day 7
100 (unmodified sample)	8.4	–
Control	8.2	8.4
1.52	8.2	8.4
3.03	-	-
6.06	-	-
12.13	8.2	8.6
24.25	-	-
48.5	-	-
97.0	8.3	8.9

"–" = not required

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519 [Program on disk and printed User's Guide].

Date : ______________________
yyyy-mm-dd

Approved By: ______________________
Project Manager

Work Order : 221257
Sample Number : 33961

Lemna minor Frond Increase

Concentration (%)	Replicate	Frond Count Day 0*	Frond Count Day 7	Frond Increase	Mean Frond Increase	Standard Deviation	CV (%)	Stimulation (%)**	Frond/Root Appearance (Day 7)
Control	A	6	97	91	90.00	9.49	10.5	–	Fronds healthy, appearance normal in all replicates.
	B	6	90	84					
	C	6	88	82					
	D	6	109	103					
1.52	A	6	108	102	102.25	12.50	12.2	–	Fronds healthy, appearance normal in all replicates.
	B	6	126	120[1]					
	C	6	100	94					
	D	6	99	93					
3.03	A	6	96	90	96.75	6.40	6.6	–	Fronds healthy, appearance normal in all replicates.
	B	6	99	93					
	C	6	106	100					
	D	6	110	104					
6.06	A	6	110	104	96.75	8.46	8.7	–	Fronds healthy, appearance normal in all replicates.
	B	6	100	94					
	C	6	92	86					
	D	6	109	103					
12.13	A	6	97	91	105.75	10.81	10.2	–	Fronds healthy, appearance normal in all replicates.
	B	6	122	116					
	C	6	117	111					
	D	6	111	105					
24.25	A	6	109	103	108.00	9.70	9.0	20.0	Fronds healthy, appearance normal in all replicates.
	B	6	120	114					
	C	6	124	118					
	D	6	103	97					
48.5	A	6	124	118	115.75	9.91	8.6	28.6	Fronds healthy, appearance normal in all replicates.
	B	6	109	103					
	C	6	121	115					
	D	6	133	127					
97.0	A	6	112	106	106.25	4.11	3.9	18.1	Fronds healthy, appearance normal in all replicates. Solutions slightly green in colour.
	B	6	107	101					
	C	6	117	111					
	D	6	113	107					

NOTES: *No unusual appearance or treatment of culture prior to testing. Test inoculated with healthy plants.
**Significant stimulation (ANOVA - Dunnett's Test (CETIS)a, α=0.05) compared to control.
•A 16.0-fold increase in frond number was observed in the control over the testing period.
• [1]Outlier according to Grubbs Test (CETIS)[a]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

"–" = not available/not required

Test Data Reviewed By : MH
Date : 2012-05-07

Work Order : 221257
Sample Number : 33961

Lemna minor Frond Weight Data

Concentration (%)	Replicate	Dry Weight of Fronds (mg)	Treatment Mean Dry Weight (mg)	Standard Deviation	Stimulation (%)**
Control	A	9.96	9.28	0.65	–
	B	8.45			
	C	9.56			
	D	9.14			
1.52	A	10.29	10.29	1.26	–
	B	12.01[2]			
	C	9.81			
	D	9.05			
3.03	A	9.00	9.63	0.61	–
	B	9.46			
	C	10.45			
	D	9.62			
6.06	A	10.08	9.57	0.42	–
	B	9.74			
	C	9.29			
	D	9.16			
12.13	A	9.67	10.39	1.11	–
	B	11.84			
	C	10.67			
	D	9.39			
24.25	A	9.94	10.63	0.83	–
	B	11.22			
	C	11.47			
	D	9.90			
48.5	A	11.84	11.99	0.90	29.18
	B	10.80			
	C	12.41			
	D	12.89			
97.0	A	10.95	11.71	0.75	26.22
	B	11.22			
	C	12.15			
	D	12.52			

NOTES : **Significant stimulation (ANOVA - Dunnett's Test (CETIS)a, α=0.05) compared to control.

• [2]Outlier according to Grubbs Test (CETIS)[a]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

"–" = not available/not required

Test Data Reviewed By : KGH
Date : 2012-05-07

AQUATOX
AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33962

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-26
Sample Description :	Cloudy, orange, odourless		

Test Method : Test for Measuring the Inhibition of Growth using the Freshwater Macrophyte, *Lemna minor*. Method Development and Application Section, Environmental Technology Centre, Environment Canada. Ottawa, Ontario. Report EPS 1/RM/37, 2nd ed. (January 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Weight)	>97.0%	-	-
IC25 (Frond Production)	>97.0%	-	-

The results reported relate only to the sample tested.

DRAFT

POTASSIUM CHLORIDE REFERENCE TOXICANT DATA

Date Tested :	Statistical Method :
Organism Batch :	Historical Geometric Mean IC25 :
Test Duration :	Warning Limits (± 2SD) :
IC25 (Frond Production) :	Growth Medium :
95% Confidence Limits :	Analyst(s) :

The reference toxicant test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

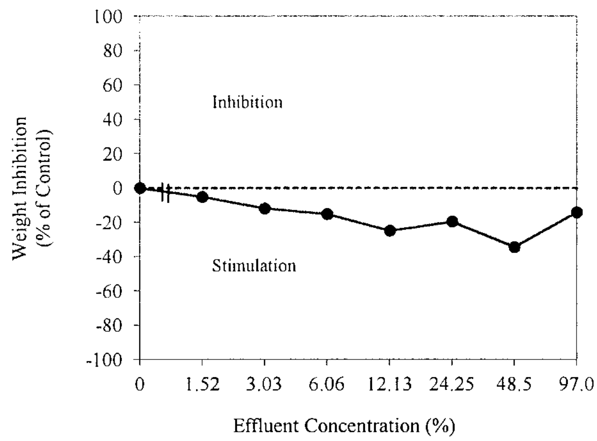
Test Organism :	*Lemna minor* L., Strain 7730	Test Type :	Static (no sub-samples required)
Organism Batch :	Lm12-04	Control/Dilution Medium :	Modified APHA
Culture Origin :	UTCC 492	Medium Preparation Water :	Distilled Water
Test Organism Source :	Axenic in-house culture	Source of Water :	Fernbrook Springs
Culture Medium :	Modified Hoaglands E+	Medium Preparation Chemicals :	Modified APHA stocks A, B, C (10 mL/L)
Age (on Test Day 0) :	9 days	Nutrient Spiking of Sample :	Modified APHA stocks A, B, C (10 mL/L)
Health Criteria (in APHA) :	15.7-fold frond increase in 7 days	Replicates per Concentration :	4
Organism Acclimation :	22:25 h in APHA medium	Test Volume per Replicate :	100 mL
Inoculum (Test Day 0) :	2 plants (3 fronds per plant)	Test Vessel :	250 mL glass Erlenmeyer flask
Sample Filtration :	1 µm (Whatman GF/C)	Depth of Test Solution :	4.0 cm
Sample Pre-aeration :	20 min. at ≤100 bubbles/min.	Photoperiod/Light Intensity :	Continuous, 4159 - 4941 lux
pH Adjustment :	None	Test Method Deviation(s) :	None
Hardness Adjustment :	None		

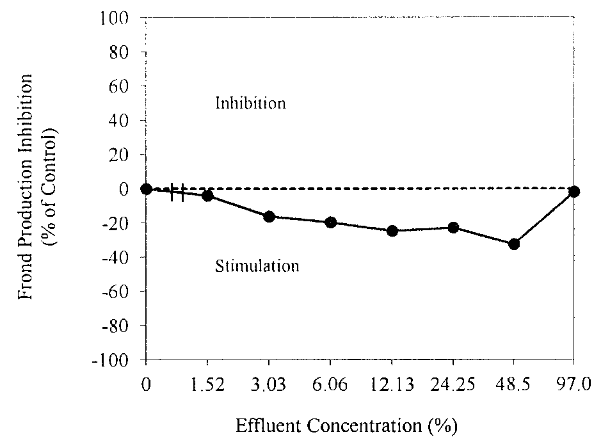
COMMENTS

•All test validity criteria as specified in the test method cited above were satisfied.

Work Order : 221257
Sample Number : 33962

Lemna minor Growth Inhibition





TEST MONITORING

Initiation Date :	2012-04-26	Termination Date :	2012-05-03
Intitiation Time :	13:40	Termination Time :	13:15
Initiated By :	SM	Terminated By :	KEH/RD

Temperature Monitoring

Test Day	Date	Temperature (°C)
0 (unmodified sample)	2012-04-26	25.0
0	2012-04-26	26.0
1	2012-04-27	26.0
2	2012-04-28	26.5
3	2012-04-29	25.5
4	2012-04-30	26.0
5	2012-05-01	26.0
6	2012-05-02	26.0
7	2012-05-03	25.5

pH Monitoring

Concentration (%)	Day 0	Day 7
100 (unmodified sample)	8.5	–
Control	8.2	8.4
1.52	8.2	8.4
3.03	-	-
6.06	-	-
12.13	8.3	8.6
24.25	-	-
48.5	-	-
97.0	8.4	8.8

"–" = not required

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville, Calif. 95519 [Program on disk and printed User's Guide].

Date : ______________________
yyyy-mm-dd

Approved By: ______________________
Project Manager

Work Order : 221257
Sample Number : 33962

Lemna minor Frond Increase

Concentration (%)	Replicate	Frond Count Day 0*	Frond Count Day 7	Frond Increase	Mean Frond Increase	Standard Deviation	CV (%)	Stimulation (%)**	Frond/Root Appearance (Day 7)
	A	6	95	89					
Control	B	6	96	90	94.50	5.80	6.1	–	Fronds healthy, appearance normal in
	C	6	105	99					all replicates.
	D	6	106	100					
	A	6	102	96					
1.52	B	6	117	111	98.25	13.67	13.9	–	Fronds healthy, appearance normal in
	C	6	86	80					all replicates.
	D	6	112	106					
	A	6	108	102					
3.03	B	6	120	114	109.75	16.01	14.6	–	Fronds healthy, appearance normal in
	C	6	99	93					all replicates.
	D	6	136	130[1]					
	A	6	126	120					
6.06	B	6	124	118	113.00	7.70	6.8	19.6	Fronds healthy, appearance normal in
	C	6	109	103					all replicates.
	D	6	117	111					
	A	6	111	105					
12.13	B	6	124	118	117.75	9.29	7.9	24.6	Fronds healthy, appearance normal in
	C	6	133	127					all replicates.
	D	6	127	121					
	A	6	112	106					
24.25	B	6	135	129	116.00	9.56	8.2	22.8	Fronds healthy, appearance normal in
	C	6	121	115					all replicates.
	D	6	120	114					
	A	6	132	126					
48.5	B	6	131	125	125.25	4.11	3.3	32.5	Fronds healthy, appearance normal in
	C	6	136	130					all replicates.
	D	6	126	120					
	A	6	93	87					
97.0	B	6	99	93	96.25	11.47	11.9	–	Fronds healthy, appearance normal in
	C	6	98	92					all replicates.
	D	6	119	113					

NOTES: *No unusual appearance or treatment of culture prior to testing. Test inoculated with healthy plants.
**Significant stimulation (ANOVA - Dunnett's Test (CETIS)a, α=0.05) compared to control.
•A 16.8-fold increase in frond number was observed in the control over the testing period.
• [1]Outlier according to Grubbs Test (CETIS)[a]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

"–" = not available/not required

Test Data Reviewed By : KEH
Date : 2012-05-08

Work Order : 221257
Sample Number : 33962

Lemna minor Frond Weight Data

Concentration (%)	Replicate	Dry Weight of Fronds (mg)	Treatment Mean Dry Weight (mg)	Standard Deviation	Stimulation (%)**
Control	A	9.18	9.12	0.17	–
	B	8.88			
	C	9.27			
	D	9.15			
1.52	A	8.85	9.58	1.14	–
	B	10.56			
	C	8.37			
	D	10.55			
3.03	A	9.51	10.20	1.40	–
	B	10.92			
	C	8.61[2]			
	D	11.74			
6.06	A	11.77	10.50	0.96	–
	B	10.03			
	C	9.55			
	D	10.64			
12.13	A	11.07	11.38	0.56	24.75
	B	10.86			
	C	11.44			
	D	12.14			
24.25	A	10.43	10.90	0.83	19.49
	B	12.13			
	C	10.42			
	D	10.61			
48.5	A	12.26	12.25	0.41	34.35
	B	12.74			
	C	11.74			
	D	12.27			
97.0	A	9.61	10.40	0.64	–
	B	11.15			
	C	10.32			
	D	10.53			

NOTES : **Significant stimulation (ANOVA - Dunnett's Test (CETIS)a, α=0.05) compared to control.

• [2]Outlier according to Grubbs Test (CETIS)[a]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

"–" = not available/not required

Test Data Reviewed By : KH
Date : 2012-05-08

AVALON RARE METALS INC.

May 10, 2012

Chronic Bioassay Test Results:
Pseodokirchneriella Subcapitata (algae)

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33960

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process Water-2	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-24
Sample Description :	Clear, colourless, odourless.		

Test Method : Growth Inhibition Test Using a Freshwater Alga. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/25, 2nd ed. (March 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth)	>90.91%	-	-

The results reported relate only to the sample tested.

ZINC (AS ZINC SULPHATE) REFERENCE TOXICANT DATA

Date Tested :	2012-04-24	Statistical Method :	Nonlinear Regression (CETIS)[a]
Organism Batch :	Ps12-04	Historical Mean IC25 :	11.0 µg/L
Test Duration :	72 hours	Warning Limits (± 2SD) :	4.3 - 28.6 µg/L
IC25 Growth :	11.5 µg/L	Analyst(s) :	VC/SM

95% Confidence Limits : 7.9 - 15.5 µg/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

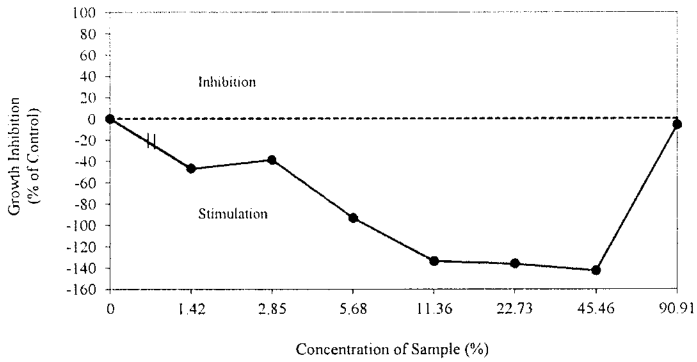
Test Organism :	*Pseudokirchneriella subcapitata*	Control/Dilution Water :	Millipore Milli-Q (no chemicals added)
Organism Batch :	Ps12-04	Test Vessel :	U-shaped polystyrene microplate
Strain Number :	UTEX (1648)	Volume per Replicate :	220 µL
Source :	In-house culture	Number of Control Replicates:	10
Culture Origin :	University of Texas, Austin TX, USA	Number of Test Replicates :	4
Age (at start of test) :	4 days (in exponential growth)	Concentrations Tested :	10 + Control
pH Adjustment :	None	Photoperiod / Light Intensity :	Continuous light, 4196 - 4368 lux
Hardness Adjustment :	None	Mean Test Temperature (± SD):	23.0°C (± 0.0)
Sample Pre-aeration :	None	Test Duration :	72 hours
Sample Filtration :	0.45 µm preconditioned filter	Test Method Deviation(s) :	None
Volume Filtered:	≥10 mL		

COMMENTS

- All test validity criteria as specified in the test method cited above were satisfied.
- No unusual appearance or treatment of culture prior to testing.
- Algal growth curve is determined at least twice per year as required by the test method cited above.

Work Order : 221257
Sample Number : 33960

***Pseudokirchneriella subcapitata* Growth Inhibition**



CELL ENUMERATION AT 72-HOURS

Initiation Date :	2012-04-24	Sample pH (at 0 hours) :	7.3
Initiated By :	VC/SM	Control pH (at 0 hours) :	6.5
Completion/Enumeration Date :	2012-04-27	Control pH (at 72 hours) :	7.0
Enumerated By :	KEH	Initial Cell Density at 0-h :	10636 cells/mL per microplate well
Enumeration Technique :	Manual (hemocytometer)	Inoculum Prepared :	00:35 h prior to test initiation
Control Cell Increase Factor :	37.1 times growth		

Concentration	Cell Concentration (x 10000 cells/mL)								Cell Yield (x 10000 cells/mL)			
	Replicate								Mean	Standard Deviation	CV (%)	Stimulation (% of control)**
(%)	1	2	3	4	7	8	9	10				
Control	41.5	44.5	35.5	40.5	40.5	43.5	35.0	35.0	38.44	3.85	10.00	–
0.18	–	–	–	–					–	–	–	–
0.35	–	–	–	–					–	–	–	–
0.71	–	–	–	–					–	–	–	–
1.42	65.5	49.5	57.5	–					56.44	8.00	14.2	46.8
2.85	54.5	53.0	55.5	–					53.27	1.26	2.4	–
5.68	79.0	74.5	72.5	–					74.27	3.33	4.5	93.2
11.36	76.5	90.5	105.5	–					89.77	14.50	16.2	133.6
22.73	76.5	94.5	104.5	–					90.77	14.19	15.6	136.2
45.46	82.0	105.5	95.5	–					93.27	11.79	12.6	142.7
90.91	45.0	51.5	29.0	–					40.77	11.58	28.4	–

NOTES : **Significant stimulation compared to control, according to ANOVA - Dunnett's Test (CETIS)[a], α=0.05.
- Control replicates 5 and 6 used for pH measurement.
- The Mann-Kendall test shows that there is no inhibitory gradient (α=0.05).
- No outlying data points were detected according to Grubbs Test (CETIS)[a].

"–" = not enumerated/not required

Data Reviewed By : JL
Date : 2012-05-03

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville CA 95519. [Program on disk and printed User's Guide].

Date : ______________ *Approved By :* ______________
yyyy-mm-dd *Project Manager*

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33961

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	PP-22 BZ-MP Treated Process + Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	B. Bowman	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-24
Sample Description :	Clear, orange, odourless		

Test Method : Growth Inhibition Test Using a Freshwater Alga. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/25, 2nd ed. (March 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth)	>90.91%	-	Linear Interpolation (CETIS) a

The results reported relate only to the sample tested.

ZINC (AS ZINC SULPHATE) REFERENCE TOXICANT DATA

Date Tested :	2012-04-24	Statistical Method :	Nonlinear Regression (CETIS)[a]
Organism Batch :	Ps12-04	Historical Mean IC25 :	11.0 µg/L
Test Duration :	72 hours	Warning Limits (± 2SD) :	4.3 - 28.6 µg/L
IC25 Growth :	11.5 µg/L	Analyst(s) :	VC/SM
95% Confidence Limits :	7.9 - 15.5 µg/L		

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

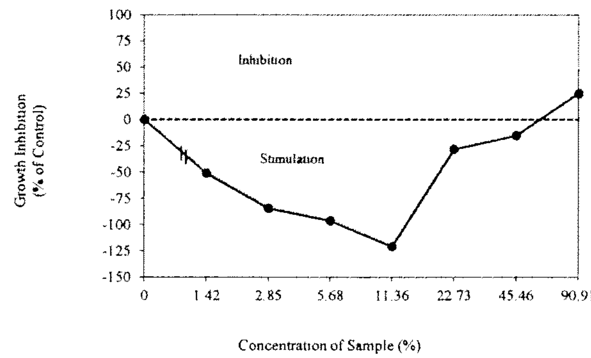
Test Organism :	*Pseudokirchneriella subcapitata*	Control/Dilution Water :	Millipore Milli-Q (no chemicals added)
Organism Batch :	Ps12-04	Test Vessel :	U-shaped polystyrene microplate
Strain Number :	UTEX (1648)	Volume per Replicate :	220 µL
Source :	In-house culture	Number of Control Replicates:	10
Culture Origin :	University of Texas, Austin TX, USA	Number of Test Replicates :	4
Age (at start of test) :	4 days (in exponential growth)	Concentrations Tested :	10 + Control
pH Adjustment :	None	Photoperiod / Light Intensity :	Continuous light, 4196 - 4368 lux
Hardness Adjustment :	None	Mean Test Temperature (± SD):	23.0°C (± 0.0)
Sample Pre-aeration :	None	Test Duration :	72 hours
Sample Filtration :	0.45 µm preconditioned filter	Test Method Deviation(s) :	None
Volume Filtered:	≥10 mL		

COMMENTS

- Statistical analysis could not be performed using non linear regression, since a suitable model could not be found. Therefore, test results were calculated using Linear Interpolation (CETIS)[a]. In test concentrations where cell yield was stimulated (greater than the control), data were replaced with control values for the purposes of statistical analysis, as recommended by Environment Canada (2005).
- All test validity criteria as specified in the test method cited above were satisfied.
- No unusual appearance or treatment of culture prior to testing.
- Algal growth curve is determined at least twice per year as required by the test method cited above.

Work Order : 221257
Sample Number : 33961

Pseudokirchneriella subcapitata Growth Inhibition



CELL ENUMERATION AT 72-HOURS

Initiation Date :	2012-04-24	Sample pH (at 0 hours) :	8.3
Initiated By :	VC/SM	Control pH (at 0 hours) :	6.5
Completion/Enumeration Date :	2012-04-27	Control pH (at 72 hours) :	6.5
Enumerated By :	AS	Initial Cell Density at 0-h :	10636 cells/mL per microplate well
Enumeration Technique :	Manual (hemocytometer)	Inoculum Prepared :	00:45 h prior to test initiation
Control Cell Increase Factor :	28.9 times growth		

Concentration (%)	Cell Concentration (x 10000 cells/mL) Replicate 1	2	3	4	7	8	9	10	Cell Yield (x 10000 cells/mL) Mean	Standard Deviation	CV (%)	Stimulation (% of control)**
Control	35.0	29.0	29.5	37.5	28.0	34.5	25.5	26.5	29.62	4.40	14.85	–
0.18	–	–	–	–					–	–	–	–
0.35	–	–	–	–					–	–	–	–
0.71	–	–	–	–					–	–	–	–
1.42	49.5	48.0	40.0	–					44.77	5.11	11.4	51.1
2.85	57.0	52.5	57.5	–					54.60	2.75	5.0	84.3
5.68	54.0	64.0	59.5	–					58.10	5.01	8.6	96.1
11.36	71.0	55.5[1]	73.0	–					65.44	9.58	14.6	120.9
22.73	35.0	40.5	41.5	–					37.94	3.50	9.2	–
45.46	35.0	40.5	30.0	–					34.10	5.25	15.4	–
90.91	23.0	24.0	23.0	–					22.27	0.58	2.6	–

NOTES : **Significant stimulation compared to control, according to ANOVA - Dunnett's Test (CETIS)a, α=0.05.
•Control replicates 5 and 6 used for pH measurement.
•The Mann-Kendall test shows that there is no inhibitory gradient (α=0.05).
•Negative cell yield indicates no growth.
• [1]Outlier according to Grubbs Test (CETIS)[a]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

2012-04-27: Algal cells appear slightly deformed, yellow, and spherical in shape in the 45.46% and 90.91% test concentrations. AS

"–" = not enumerated/not required

Data Reviewed By : JL
Date : 2012-05-09

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville CA 95519. [Program on disk and printed User's Guide].

Environment Canada. 2005. Guidance Document on Statistical Methods for Environmental Toxicity Tests. Environment Canada, Method Development and Application Section, Environmental Technology Centre, Environmental Protection Service. Ottawa, Ontario. EPS 1/RM/46, December 2005.

Date : 2012-05-09
yyyy-mm-dd

Approved By : [signature]
Project Manager

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd.
RR 3
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

Work Order : 221257
Sample Number : 33962

SAMPLE IDENTIFICATION

Company :	SGS Lakefield Research Limited		
Location :	Lakefield ON	Date Collected :	2012-04-23
Substance :	Lake Water	Time Collected :	15:50
Sampling Method :	Grab	Date Received :	2012-04-24
Sampled By :	BB	Time Received :	10:30
Temp. on arrival :	13.0°C	Date Tested :	2012-04-25
Sample Description :	Cloudy, orange, odourless		

Test Method : Growth Inhibition Test Using a Freshwater Alga. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/25, 2nd ed. (March 2007).

TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth)	>90.91%	-	-

The results reported relate only to the sample tested.

ZINC (AS ZINC SULPHATE) REFERENCE TOXICANT DATA

Date Tested :	2012-04-24	Statistical Method :	Nonlinear Regression (CETIS)[a]
Organism Batch :	Ps12-04	Historical Mean IC25 :	11.0 µg/L
Test Duration :	72 hours	Warning Limits (± 2SD) :	4.3 - 28.6 µg/L
IC25 Growth :	11.5 µg/L	Analyst(s) :	VC/SM

95% Confidence Limits : 7.9 - 15.5 µg/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST CONDITIONS

Test Organism :	*Pseudokirchneriella subcapitata*	Control/Dilution Water :	Millipore Milli-Q (no chemicals added)
Organism Batch :	Ps12-04	Test Vessel :	U-shaped polystyrene microplate
Strain Number :	UTEX (1648)	Volume per Replicate :	220 µL
Source :	In-house culture	Number of Control Replicates:	10
Culture Origin :	University of Texas, Austin TX, USA	Number of Test Replicates :	4
Age (at start of test) :	5 days (in exponential growth)	Concentrations Tested :	10 + Control
pH Adjustment :	None	Photoperiod / Light Intensity :	Continuous light, 4196 - 4368 lux
Hardness Adjustment :	None	Mean Test Temperature (± SD):	23.0°C (± 0.0)
Sample Pre-aeration :	None	Test Duration :	72 hours
Sample Filtration :	0.45 µm preconditioned filter	Test Method Deviation(s) :	None
Volume Filtered:	≥10 mL		

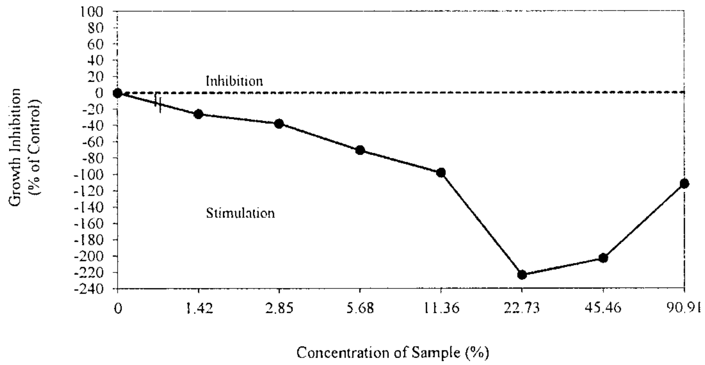
COMMENTS

- All test validity criteria as specified in the test method cited above were satisfied.
- No unusual appearance or treatment of culture prior to testing.
- Algal growth curve is determined at least twice per year as required by the test method cited above.

AQUATOX

Work Order : 221257
Sample Number : 33962

Pseudokirchneriella subcapitata Growth Inhibition



CELL ENUMERATION AT 72-HOURS

Initiation Date : 2012-04-25
Initiated By : AS
Completion/Enumeration Date : 2012-04-28
Enumerated By : CL
Enumeration Technique : Manual (hemocytometer)
Control Cell Increase Factor : 27.7 times growth

Sample pH (at 0 hours) : 8.5
Control pH (at 0 hours) : 6.5
Control pH (at 72 hours) : 6.5
Initial Cell Density at 0-h : 10864 cells/mL per microplate well
Inoculum Prepared : 00:50 h prior to test initiation

Cell Concentration (x 10000 cells/mL) — **Cell Yield (x 10000 cells/mL)**

Concentration (%)	Replicate 1	2	3	4	7	8	9	10	Mean	Standard Deviation	CV (%)	Stimulation (% of control)**
Control	30.0	29.5	29.0	32.5	29.5	34.5	25.5	30.0	28.98	2.62	9.06	–
0.18	–	–	–	–					–	–	–	–
0.35	–	–	–	–					–	–	–	–
0.71	–	–	–	–					–	–	–	–
1.42	34.0	42.0	37.0	–					36.58	4.04	11.0	–
2.85	35.5	48.5	39.0	–					39.91	6.73	16.9	37.7
5.68	53.0	48.5	50.0	–					49.41	2.29	4.6	70.5
11.36	62.5	59.5	53.5	–					57.41	4.58	8.0	98.1
22.73	98.5	91.0	95.0	–					93.75	3.75	4.0	223.5
45.46	86.0	81.5	99.5	–					87.91	9.37	10.7	203.4
90.91	56.5	62.5	68.5	–					61.41	6.00	9.8	111.9

NOTES : **Significant stimulation compared to control, according to ANOVA - Dunnett's Test (CETIS)[a], α=0.05.
•Control replicates 5 and 6 used for pH measurement.
•The Mann-Kendall test shows that there is no inhibitory gradient (α=0.05).
•No outlying data points were detected according to Grubbs Test (CETIS)[a].

"–" = not enumerated/not required

Data Reviewed By :
Date : 2012-05-03

REFERENCES

[a] CETIS, © 2001-2007. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, McKinleyville CA 95519. [Program on disk and printed User's Guide].

Date : 2012-05-09
yyyy-mm-dd

Approved By :
Project Manager

Attachment 2

EBA Engineering Consultants Ltd. operating as EBA, A Tetra Tech Company
Oceanic Plaza, 9th Floor, 1066 West Hastings Street
Vancouver, BC V6E 3X2 CANADA
p. 604.685.0275 f. 604.684.6241

TECHNICAL MEMO

ISSUED FOR USE

TO:	Rick Hoos	**DATE:**	April 18, 2012
C:		**MEMO NO.:**	
FROM:	David Morantz	**EBA FILE:**	V15101007.004

SUBJECT: Background Water Quality Assessment – Avalon Thor Lake Project

Water Quality Summary

The table below provides a summary of the baseline water quality data for total metals in Drizzle, Murky, and Thor lakes. For comparison, the table also shows laboratory detection limits, CCME guideline levels, and the modelled values of chemical parameters in each of the lakes due to discharges from the Tailings Management Facility (TMF) for year 20 following mine startup. The year-20 value represents a worst case situation since modelled values for years one to twenty progressively increase with time due to the recycling and subsequent concentration of metals in the recycle stream. All data have been summarized from Appendix F in the Stantec (2011) Environmental Baseline Report, Volume 3-Aquatics and Fisheries.

Mean and standard deviation values in the table are based on a summary of analysis results from the following sampling periods:

Drizzle Lake: September 2009; April 2010; June 2010; September 2010; October 2010.

Murky Lake: March 2008; October 2008; March 2009; June 2009; September 2009; April 2010; June 2010; September 2010; October 2010.

Thor Lake: March 2008; October 2008; March 2009; June 2009; September 2009; April 2010; June 2010; September 2010; October 2010.

Comments

The water quality summary indicates that mean background values of all parameters, except iron in Drizzle and Murky lakes, are less than CCME guideline levels. Some metals were consistently less than the laboratory detection limits. The high mean iron values in Drizzle and Murky lakes are skewed due to very high concentrations of this metal found in samples collected under the ice in March and April (as indicated by a standard deviation that is more than twice the mean). In all cases, iron values are less than CCME guideline levels during open water periods. This can be explained by the fact that iron is released from the sediments under anoxic conditions, which exist under the ice in Drizzle and Murky lakes. Iron levels in Thor Lake did not exceed CCME guideline levels at any time during the study period.

The predicted concentrations of metals in each of the lakes during mine operation, under worst case conditions, are consistently within background levels. Based on modelling results, the operation of the mine is therefore not anticipated to affect significantly the metals concentrations in any of the lakes.

Mean and standard deviation[i] of selected baseline water quality parameters and predicted (modelled) values resulting from discharges from the Tailings Management Area in Year 20.

Parameter	CCME Guideline	Detection Limit[ii]	Drizzle Lake			Murky Lake			Thor Lake		
			Mean	S.D.	Modelled Value	Mean	S.D.	Modelled Value	Mean[iii]	S.D.	Modelled Value
Aluminum	100	5.0	8.30	9.10	10.0	7.20	3.90	8.53	3.30	0.54	3.77
Arsenic	5.0	0.1	0.92	0.23	0.93	1.29	0.51	1.29	0.77	0.06	0.77
Cadmium	0.052	0.017	0.01	0.002	0.01	0.02	0.01	0.02	0.02	0.01	0.02
Chromium	8.9	0.5	0.25	0	0.25	0.25	0	0.25	0.28	0.02	0.28
Copper	2-4	0.3	0.25	0.09	0.26	0.36	0.13	0.36	0.36	0.15	0.36
Iron	300	30	1091	2322	1093	3054	4948	3055	69.50	25.40	69.93
Lead	1-7	0.05	0.028	0.01	0.030	0.03	0.01	0.031	0.05	0.05	0.050
Mercury	0.026	0.05	0.005	0	0.005	0.01	0.01	0.01	0.01	0	0.01
Molybdenum	73	0.05	1.27	0.48	1.40	1.42	0.53	1.52	2.10	0.03	2.14
Nickel	25-150	0.5	0.25	0	0.27	0.25	0	0.27	0.25	0	0.26
Selenium	1.0	1.0	0.50	0	0.50	0.50	0	0.50	0.50	0	0.50
Silver	0.1	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0	0.01
Thallium	0.8	0.1	0.05	0	0.05	0.05	0	0.05	0.05	0	0.05
Zinc	30	1.0	0.90	0.60	0.92	2.30	1.10	2.32	1.43	0.50	1.44

[i] All concentration values in µg/L.

[ii] Where sample values were less than the detection limit, half the value of the detection limit was used for computations; standard deviation values of 0 imply that all measurements were less than the detection level.

[iii] Mean of mean values for four sampling locations in Thor Lake.

Attachment 3

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MEETING NOTES

ISSUED FOR USE

ATTENDEES: Nathen Richea, Paul Green – Aboriginal Affairs and Northern Development Canada
Anne Wilson – Environment Canada
Rick Hoos-EBA
David Morantz-EBA

DATE: May 1, 2012

EBA FILE: V15101007.004

MEETING TITLE: **Avalon Thor Lake Site Specific Water Quality Objectives**

The telecon meeting was held to discuss water quality issues related to the possible development of site specific water quality objectives (SSWQO) for the Avalon Rare Metals Inc. Thor Lake Project. The following summarizes the topics that were raised during the meeting:

- In advance of specific discussions related to water quality issues, EBA indicated that a complete copy of the Developer's Assessment Report (DAR) would be sent (preferably electronically) to Nathen and Anne, since the MVEIRB Registry website provides sections of the DAR as separate files. At the end of the call, EBA indicated that all necessary tables from various documents and IR responses would be provided as part of the meeting notes, for submission to the MVEIRB.
- Some questions were raised concerning tabular data that was recently sent in the April 18, 2012 memo to AANDC and EC, regarding baseline and modeled WQ information for Drizzle, Murky, and Thor lakes. In particular, it was noted that natural seasonal baseline iron levels were shown to be considerably higher than Canadian Council of Ministers of the Environment (CCME) guideline limits for the protection of aquatic life. Also, the reported iron concentrations were quite variable, which produced a very large standard deviation (twice the value of the mean).

  EBA indicated that the mean values for iron were skewed due to very high concentrations found under the ice in winter, due to anoxic conditions. The data presented as an Appendix to the DAR showed that such high values only occurred in winter in shallow lakes, where anoxic conditions resulted in the mobilization of iron from the substrate. EBA further suggested that SSWQOs should not be related to natural extreme events, rather they should be related to more normal concentrations measured during the open water period.
- AANDC made an inquiry into what the worst case scenario presented in the Table provided in the April 18, 2012 memo represented – EBA identified that the worst case referred to the predicted effluent quality after 20 years of operation. These values did not consider any influence of wet years or dry years. The values presented in the April 18 Table represented the expected water quality within the entire lake at Year 20.
- It was questioned whether the estimated discharge rate and quality (from the Tailings Management Facility (TMF), polishing pond, or processing plant) shown in the DAR are likely to change. In response, EBA indicated that the design of the TMF and the operation were continuing to be

optimized in the current feasibility study and that such refinements are anticipated to result in further improvements, in particular possible reductions in the decant discharge volumes.

- There was discussion concerning nutrient (nitrate, phosphorous, etc.) discharges from the TMF, which were not included in the recent (April 18) table of baseline and modeled water quality parameters that was sent to AANDC and EC. It was indicated that information regarding nutrient concentrations in the effluent has been provided in responses to specific AANDC and EC Information Requests, which indicated that phosphorous levels in treated sewage discharged to the TMF will be very low due to the state-of-the-art treatment facility that will be used at the mine site. However, it was agreed that a summary of information concerning phosphorous levels in the mine discharge will be provided by the end of April.

- There was a discussion about the lack of information on the background concentrations of Rare Earth Elements (REE). EBA indicated that further groundwater sampling is currently being conducted, which will provide information on background REE concentrations. The expected delivery date for this sampling event is the end of April, 2012. In addition, chronic toxicity analyses are presently being run using standard EC bioassay tests. The results, including characterization of the effluent used in the tests, will be provided to the MVEIRB, AANDC and EC when they become available (acute results are expected at the end of April). These results, including the chronic toxicity information, which is not expected for a few more weeks, will contribute to the consideration of site specific water quality objectives.

- Table 1 of the February 20 memo (Response to AANDC IR #2.1) presented data which indicated that Cerium and Lanthanum concentrations in the 5 Day Decant may be potentially toxic to Hyallela and Rainbow Trout (lanthanum only), based on a review of U.S. Environmental Protection Agency information. However, EBA indicated that these data are very conservative and reflect worst case conditions since they were based on toxicity tests using dissolved Rare Earth Metals (REE) in waters having low hardness concentrations (toxicity is inversely related to hardness; the hardness of water in the Thor Lake drainage is considerably higher than in water used for the reported toxicity tests).

  Toxicity information for REEs are contained in a comprehensive document prepared by Wilfred Laurier University. EBA agreed to provide a digital copy of this document to AANDC and EC. It was noted by EBA that this report indicates that REEs generally have a low solubility and low toxicity levels. AANDC agreed to review this new document and get back to EBA is they had additional questions.

- Additional discussion occurred regarding chronic toxicity and its relationship to mixing zones. AANDC acknowledged that Avalon has suggested using Drizzle Lake as the mixing zone. It was noted that Drizzle Lake is very shallow and has not been shown to support fish. Modeled values for metals in Drizzle Lake in year 20 of mine operation are considerably lower than the existing CCME guidelines, except for iron in winter. These predicted concentrations may form the basis for discussions regarding the setting of SSWQOs for this project. There was some discussion whether CCME guideline levels are appropriate as SSWQOs. AANDC noted CCMEs non-degradation approach and pollution prevention principles in the recent Land and Water Board Water and Effluent Quality Policy. Environment Canada noted that CCME guidelines do not exist for the REEs.

- AANDC indicated that the outlet of Drizzle Lake may be the appropriate location for establishing the discharge point for monitoring of SSWQOs. It was noted that the water quality model indicates that effluent will be of relatively high quality.
- Discussion then moved to Environmental Effects Monitoring (EEM), which is mandated by the Metal Mining Effluent Regulations (MMER). AANDC noted that EEM monitoring would be required as a minimum but that an even more rigorous monitoring program may be required per its AEMP Guidelines.
- Although AANDC has noted that Avalon has requested to use CCME Guidelines as the primary basis for establishing SSWQOs at the outlet of Drizzle Lake, it was concerned that CCME Water Quality Guidelines do not exist for all parameters of concern. AANDC also indicated that given the predicted high quality of the decant discharge from the Nechalacho Tailings Management Facility to the downstream receiving environment, the adoption of CCME criteria for the establishment of SSWQOs for this project could be a good initial starting point for further discussions.
- Discussions concluded with a consensus that the meeting was productive, and that future meetings to resolve issues related to the setting of SSWQOs for other parameters for which CCME criteria do not yet exist would be valuable, particularly when the results of REEE and acute toxicity were available (end of April).

Attached to this Technical Memo are copies of the Tables of predicted effluent/downstream water quality that were previously provided in response to various AANDC and EC Information Requests.

Table 1 is excerpted from the February 2012 Avalon response to AANDC Information Request #2:

TABLE 1: COMPARISON OF PREDICTED RARE EARTH ELEMENT CONCENTRATIONS WITH AVAILABLE TOXICITY DATA – THOR LAKE

Element	Day 5 Decant Concentration in Tailings Discharge (mg/L)	Max. Predicted Concentration Years 1-20 (mg/L)	LC 50 Concentration (mg/L)	Aquatic Organism Affected
Cerium (Ce)	1.39E-1	1E-4	0.032	*Hyalella Azteca* (amphipod crustacean)
Dysprosium (Dy)	2.52E-3	1.9E-6	0.162	*Hyalella Azteca*
Erbium (Er)	5.81E-4	4.4E-7	0.191	*Hyalella Azteca*
Europium (Eu)	1.09E-3	8.3E-7	0.112	*Hyalella Azteca*
Gallium (Ga)	2.86E-3	2.2E-6	>1.0	*Hyalella Azteca*
Gadolinium (Gd)	9.37E-3	7.1E-6	0.150	*Hyalella Azteca*
Holmium (Ho)	3.12E-4	2.4E-7	n/a	
Lanthanum (La)	6.88E-2	5.2E-5	0.018 0.020	*Hyalella Azteca* *Oncorhynchus mykiss* (Rainbow trout)
Lutetium (Lu)	3.3E-5	2.5E-8	0.029	*Hyalella Azteca*
Niobium (Nb)	2.57E-3	1.9E-6	0.026	*Hyalella Azteca*
Neodymium (Nd)	6.16E-2	4.7E-5	0.143	*Hyalella Azteca*
Praseodymium (Pr)	1.73E-2	1.3E-5	0.035	*Hyalella Azteca*
Scandium (Sc)	3.39E-3	2.6E-6	0.029	*Hyalella Azteca*
Samarium (Sm)	1.10E-2	8.3E-6	0.074	*Hyalella Azteca*
Tantalum (Ta)	2.30E-4	1.7E-7	0.002	*Hyalella Azteca*
Terbium (Tb)	8.19E-4	6.2E-7	0.084	*Hyalella Azteca*
Thulium (Tm)	4.6E-5	3.5E-8	n/a	
Ytterbium (Yb)	3.24E-4	2.5E-7	0.069	*Hyalella Azteca*
Zirconium (Zr)	3.29E-3	2.5E-6	>1.0	*Hyalella Azteca*

Table 3 is excerpted from the February 2012 Avalon response to AANDC IR #6.2/3:

TABLE 3: MAXIMUM CONCENTRATION IN THE THOR LAKE SYSTEM AND WATER QUALITY GUIDELINES FOR THE PARAMETERS OF CONCERN

Species	Background Concentration			Thor Lake	Murky Lake	Drizzle Lake	CCME Water Quality Guideline	MMER Effluent Criteria
	Thor Lake	Murky Lake	Drizzle Lake					
TDS (mg/L)	191	224	169	191.3	224.9	170.1	-	-
Cl (mg/L)	4.35	5.30	3.60	4.39	5.39	3.72	-	-
SO_4 (mg/L)	0.28	0.30	< 0.5	0.351	0.514	< 0.771	-	-
F (mg/L)	1.11	1.04	0.893	1.11	1.05	0.91	-	-
NO_3* (as N mg/L)	0.053	0.014	-	0.053	0.014	0.014	2.9	-
Ammonia (as N mg/L)	< 0.02	0.70	0.71	< 0.02	0.70	0.71	-	-

The background concentration value for NO_3 in Drizzle Lake was missing and assumed to have the same value as the background concentration in Murky Lake.

Table 20 is excerpted from the January 2012 Avalon response to the Environment Canada IR #13.1.

TABLE 20: NECHALACHO FLOTATION PLANT TAILINGS AND TMF DECANT COMPOSITION

Tailings Solids Component[2]		
Parameter	Units	PP1 Tls
SiO_2	%	60.2
Al_2O_3	%	13.2
Fe_2O_3	%	10.7
MgO	%	2.43
CaO	%	0.85
Na_2O	%	3.35
K_2O	%	6.05
TiO_2	%	0.03
$P2O_5$	%	0.04
MnO	%	0.09
Cr_2O_3	%	<0.01
$V2O_5$	%	<0.01
LOl	%	1.54
Sum	%	98.5
Nb_2O_5	%	0.18
ZrO_2	%	1.52

Parameter	Units	Tailings Liquid Component[1,2]	PP1 Tls Decant Day 5[2]	Regulations and Guidelines	
		PP1 Tls	PP1 Tls	MMER	CCME Guideline for the Protection of Aquatic Life
Initial pH	units	9.28			
Final pH	units	8.81			
Radionuclide Analyses					
226Ra	Bq/L	<0.01	<0.01	0.37	
228Ra	Bq/L	<0.3	0.3		
210Pb	Bq/L	<0.1	<0.1		
General and Metals Analyses					
pH	units	7.95	8.20		6.5-9.0
Alkalinity	mg/L as $CaCO_3$		119		
EMF	mV		284		
Conductivity	µS/cm		617		
TDS	mg/L		400		
TSS	mg/L		14		
Cl	mg/L	3.6	44		
SO_4	mg/L		100		
F	mg/L	1.83	4.43		
TOC	mg/L		12.2		
Hg	mg/L	<0.0001	<0.0001		0.000026
As	mg/L	0.0199	0.0022	0.5	0.005
Ca	mg/L	21.0	43.7		
Cu	mg/L	0.0010	0.0023	0.30	Minimum[3] 0.002

Table 20 continued…

		Parameter	Units	Tailings Liquid Component[1,2]	PP1 Tls Decant Day 5[2]	Regulations and Guidelines	Parameter
				PP1 Tls	PP1 Tls	MMER	CCME Guideline for the Protection of Aquatic Life
		Fe	mg/L	0.041	0.570		0.3
		K	mg/L	8.76	28.8		
		Mg	mg/L	3.20	9.14		
		Mn	mg/L		0.0788		
		Na	mg/L	13.4	70.4		
		Ni	mg/L	0.0059	0.0070	0.50	Minimum[3] 0.025
		Pb	mg/L		0.00060	0.20	Minimum[3] 0.001
		Se	mg/L	<0.001	<0.001		0.001
		Si	mg/L	4.72	8.10		
		Th	mg/L	0.000832	0.000694		
		U	mg/L	0.00535	0.00880		0.015
		Y	mg/L		0.00877		
		Zn	mg/L	0.003	0.007	0.50	0.03

1. CCME guidelines are not typically applied to tailings facilities.
2. Source: SGS Minerals Services. August 30, 2011. Environmental Characterisation of Ore, Concentrate and Tailings from the Nechalacho Rare Earth Element Project – Phase #2. Prepared for Avalon Rare Metals Inc. (Project 11806-007) (Tables 10, 20, and 24)
3. The minimum guidelines have been expressed in this table. The CWQG copper and lead equations determine specific guidelines based on water hardness. [**copper concentration guidelines =** $e^{0.8545[\ln(hardness)]-1.465}$ *** 0.2 µg/L;** nickel concentration guidelines = $e^{0.76[\ln(hardness)]+1.06}$ µg/L; **lead concentration guidelines =** $e^{1.273[\ln(hardness)]-4.705}$ **µg/L.]**

The following table is excerpted from the April 18, 2012 memo addressed to Anne Wilson and Nathen Richea.

Mean and standard deviation[i] of selected baseline water quality parameters and predicted (modelled) values resulting from discharges from the Tailings Management Area in Year 20.

Parameter	CCME Guideline	Detection Limit[ii]	Drizzle Lake			Murky Lake			Thor Lake		
			Mean	S.D.	Modelled Value	Mean	S.D.	Modelled Value	Mean[iii]	S.D.	Modelled Value
Aluminum	100	5.0	8.30	9.10	10.0	7.20	3.90	8.53	3.30	0.54	3.77
Arsenic	5.0	0.1	0.92	0.23	0.93	1.29	0.51	1.29	0.77	0.06	0.77
Cadmium	0.052	0.017	0.01	0.002	0.01	0.02	0.01	0.02	0.02	0.01	0.02
Chromium	8.9	0.5	0.25	0	0.25	0.25	0	0.25	0.28	0.02	0.28
Copper	2-4	0.3	0.25	0.09	0.26	0.36	0.13	0.36	0.36	0.15	0.36
Iron	300	30	1091	2322	1093	3054	4948	3055	69.50	25.40	69.93
Lead	1-7	0.05	0.028	0.01	0.030	0.03	0.01	0.031	0.05	0.05	0.050
Mercury	0.026	0.05	0.005	0	0.005	0.01	0.01	0.01	0.01	0	0.01
Molybdenum	73	0.05	1.27	0.48	1.40	1.42	0.53	1.52	2.10	0.03	2.14
Nickel	25-150	0.5	0.25	0	0.27	0.25	0	0.27	0.25	0	0.26
Selenium	1.0	1.0	0.50	0	0.50	0.50	0	0.50	0.50	0	0.50
Silver	0.1	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0	0.01
Thallium	0.8	0.1	0.05	0	0.05	0.05	0	0.05	0.05	0	0.05
Zinc	30	1.0	0.90	0.60	0.92	2.30	1.10	2.32	1.43	0.50	1.44

i All concentration values in µg/L.

ii Where sample values were less than the detection limit, half the value of the detection limit was used for computations; standard deviation values of 0 imply that all measurements were less than the detection level.

iii Mean of mean values for four sampling locations in Thor Lake.

Attachment 4

Review of Aquatic Effects of Lanthanides and Other Uncommon Elements

Final Project Report

March 31, 2011

Prepared for the

EC Contribution Agreement with the CNTC for 2010/2011

Tania Ng, D. Scott Smith, Anthony Straus and James C. McGeer

Wilfrid Laurier University

Table of Contents

Summary 3

Background and Overall Objectives: 4

Progress Against Milestones for February 1, 2010 - March 31, 2011: 4

Sources of Lanthanides 5

Concentrations in the Environment 6

Solubility & Speciation 7

Potential Precipitates in Toxicity Tests 8

Geochemical Speciation 9

Bioavailability 9

Biological Effects on Aquatic Organisms 10

i). Acute Toxicity 10

ii). Chronic Toxicity 12

Bioaccumulation by Aquatic Organisms 13

Data Gaps & Recommendations for Toxicity Testing 15

i). Test Elements 15

ii). Test Systems 15

iii). Test Species 15

iv). Test Methods 16

v). Test Topics 16

References 35

Appendix A. 39

Project Final Report

Reporting Period: Feb. 7 – Mar. 31, 2011

Summary

Project Title: Review of Aquatic Effects of Lanthanides & Other Uncommon Elements

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MILESTONE DESCRIPTIONS	MILESTONES PROGRESS
1. Bibliographic listing of primary or secondary literature on lanthanides to March 31, 2011, with annotations.	Completed. In year 1, Dr. Gheorghiu has searched a list of literature articles on lanthanides. In year 2, Dr. Ng added “bioavailability” to the search items and included secondary literature articles on the list. A total of 6359 papers were found from the search. Of these, 629 papers, with annotations (complete author listing, abstracts, source) were retained for review. Search strategies and results are summarized in Table 1. The complete reference list, in annotated form, is also attached.
2. Establish an EndNote data-base on the ecotoxicology of lanthanides.	Completed. A comprehensive database comprising 629 peer-reviewed papers and some government reports has been compiled. Articles were categorized into groups using keywords: bioaccumulation, bioavailability, birds, fish, invertebrates, mammals, plants, solubility, speciation, toxicity. The EndNote library is attached as a separate file.
3. Full review of the lanthanides literature, evaluation of data, identification of data gaps, and recommendations for aquatic ecotoxicity testing program.	Completed. In year 2, articles from the EndNote database were critically reviewed. Data are collected for evaluation on sources (Table 2); concentrations in the environment (Fig. 1); speciation & solubility (Table 3, Fig 2,3); geochemical speciation; bioavailability; toxicity (Table 4 – 6) and bioaccumulation of lanthanides (Table 7 - 8).

Final Report: Review of Aquatic Effects of Lanthanides & Other Uncommon Elements

Background and Overall Objectives:

The lanthanides (also called Rare Earth Elements – REE, or Lanthanoids) are the chemical elements found in Row 6 of the periodic table between Groups 3 and 4, beginning with lanthanum (La), which accounts for the group name. The lanthanides consist of the following metals: lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu).

The present knowledge concerning the eco-toxicological effects of lanthanides is limited, with few studies having addressed the biological effects of the lanthanides on aquatic plants and animals. The **overall objective** of this project is to collect, collate and review the existing literature on the eco-toxicity of the lanthanide elements. The compiled a database on the toxicology of the lanthanides was reviewed with the goal of identifying gaps in our understanding on the chemistry, bioavailability, and toxicity potential of the lanthanides to aquatic organisms.

Progress Against Milestones for February 1, 2010 - March 31, 2011:

A database on the lanthanides (Milestone 1) was established by conducting literature searches using the PubMed [(US National Library of Medicine (NLM), National Institute of Health (NIH)], ISI Web of Science (Thomson Reuters) and TOXNET (NLM-NIH) databases. Searches on the lanthanides in general, and the individual lanthanides, were conducted using the following keywords: rare earth elements, lanthanides, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium. Searches were then narrowed using these keywords in combination with the additional terms: ecotoxicity, environmental toxicity, toxicity, speciation, bioaccumulation, fish, invertebrates, plants. This search was completed in phase 1 of the project which was led by

Wilkie et al. and delivered in March 2010. This search was updated at the beginning of phase 2 (February 2011). Since "bioavailability" is a key topic area of interest, it was added as one of the search keywords in year 2. Web of Science was only used to run this search because based on previous search experience it provides the maximum number of relevant articles in aquatic science. Results for each search combination are presented in Table 1. From these searches, there were a total of 6,359 "hits" requiring further screening, which led to the identification of 862 articles. These were assessed for availability and retained for further screening. After further screening, and elimination of duplicates, a total of 577 peer-reviewed published articles and government reports plus 52 additional articles (primary and secondary – not accessible or written in other languages) cited in literature, resulting to a total of 629 articles entered into an EndNote database (Milestone 2; library attached). The smart group feature of EndNote was used to generate subfolders for the following topics: speciation, bioaccumulation, bioavailability, birds, fish, invertebrates, mammals, plants, solubility, speciation, toxicity. A complete annotated reference list (authors, year, abstracts, journal) of the articles in the EndNote database is attached with the report. Articles where an electronic version (pdfs) was available were used for a critical review of: sources; concentrations in the environment; solubility, speciation and partitioning; bioavailability; toxicity; and bioaccumulation of lanthanides (Milestone 3; summary of evaluation provided below).

Sources of Lanthanides

Contrary to their designation as rare earth elements, the lanthanides are relatively abundant in the Earth's crust (except for promethium, a radioactive artificial element which is a byproduct of spontaneous uranium decay). REEs are usually found together in minerals (Table 2) and about 160 mineral ores are known to contain REE at levels of up to 60 % (Slooff et al., 1993). Approximately 80 % of known REE mineral supplies/desposits are found in China (Annema, 1990). Highly pure, low-cost rare earths can be industrially produced mainly through ion-exchange and solvent extraction processes. Lanthanides are used in different industries, most commonly as petroleum refining catalysts or catalytic converters, as permanent magnets; in glass polishing and ceramics, the production of sunglass lenses; laser industries; as chemical fertilizers, or trace supplements in agriculture; as misch metal to remove oxygen and to enrich steel; as luminophores; and as high-temperature superconductors (Palasz and Czekaj, 2000). In addition, radioisotopes of lanthanides are used in the anticancer therapy (Alberts et al., 1997).

Concentrations in the Environment

Due to its co-existence with other minerals, natural concentration of lanthanides are similar to essential elements such as iodine, cobalt and selenium (Palasz and Czekaj, 2000). Compared to the non-essential metals, concentrations of La, Ce and Nd tend to be higher than Pb and even concentrations of the less abundant REEs, (e.g. Tm and Lu), are typically higher than Cd in the earth crust (Weltje, 2002). An exception is Pm, a by-product from decay of uranium and not naturally present in the earth crust. Natural concentrations of other REEs in the earth crust display a typical saw-tooth pattern with a log-linear decrease of concentration with atomic number (Z). Additionally, the odd-numbered elements have lower concentrations than the even-numbered elements (Weltje, 2002) (Fig. 1). In general, the highest concentration is found for Ce, the first even-numbered lanthanide, while the lowest concentrations are found for the last two odd-numbered lanthanides, Tm and Lu, whose concentrations are about two orders of magnitude lower than those of Ce.

REEs have low solubility (see "Solubility &, Speciation" section) and dissolved (passing ≤ 0.45 μm filter) Ce and Lu only ranged from 2.9 – 714 ng L^{-1} and 0.04 – 7 ng L^{-1} respectively in the pristine freshwater environments (Weltje, 2002). Dissolved concentrations of other REEs are between these ranges. Pore water often has slightly higher dissolved REEs than surface water due to its lower water pH that increases solubility (Weltje et al., 2002b). In contrast to dissolved REEs, concentrations in sediment are much higher (Sneller et al., 2000), reflecting the high K_p sediment-water partition coefficient (see "Geochemical Speciation" section). For example, concentrations of Ce, La, Pr and Nd in unpolluted sediment of Tsurumin River, Japan are 45.1, 22.3, 6.2 and 21.1 mg kg^{-1} respectively (Mohiuddin et al., 2010). Due to the low solubility of many inorganic lanthanide salts, the high affinity for sediment and suspended matter, and their tendency to form complexes with both inorganic and organic ligands, the free ion concentrations of lanthanides in natural freshwaters is very low (pM to nM range). Compared to freshwater sediments (0.3 – 68.9 mg kg^{-1}), background concentration of REE elements in the sediment of saltwater environments (0.3 – 92.6 mg kg^{-1}) tend to be higher (Sneller et al., 2000).

The main anthropogenic emissions of REEs are to surface water (Slooff et al., 1993) and subsequently the majority of lanthanides end up in sediment. Depositions associated with phosphate fertilizer and discharges from catalyst producing industries have resulted in contaminated sediment in the Rhine R. estuary (Netherlands) where concentrations reach up to 170 mg kg^{-1} Ce, 80 mg kg^{-1} La, 80 mg kg^{-1} Nd, 30 mg kg^{-1} Pr and 20 mg kg^{-1} Sm (Sneller et al., 2000). The lanthanum-modified clay Phoslock®, which is used to remove phosphate from wastewater in Australia, results in total La concentrations as high as 400 μg L^{-1} after one application (Flapper, 2003; McIntosh, 2007) with dissolved lanthanum concentrations being up to 12 μg L^{-1} after several applications over 24-48 h after which it then dropped rapidly to below 1 μg L^{-1} (Haghseresht, 2006).

Solubility & Speciation

Generally, solubility of REEs is low due to complex formation and the low solubility product (K_{sp}) of the complex. Dominant species of inorganic and organic lanthanide complexes in solution are given in Table 3. Often the simple fluorides, oxalates, phosphates, and carbonates are sparingly soluble while the complex carbonates, sulfates, and fluorides are readily soluble (Moeller and Vincenti, 1965; Cantrell and Byrne, 1987). Since carbonates, phosphates, hydroxides are ubiquitous ligands in aquatic environments they strongly limit aqueous lanthanide concentration. The K_{sp}'s of most complexes are very low e.g., K_{sp} of REE-phosphates can be as low as 10^{-25} mol^2 l^{-2} (Liu and Byrne, 1997); K_{sp} of REE-carbonates and hydroxides are the lowest as 10^{-30} mol^5 l^{-5} and 10^{-24} mol^4 l^{-4} at 25 ^oC respectively (Martell and Smith, 1997-2001). However, solubility of REEs is also strongly dependent on pH and temperature. Increase in temperature increases solubility. An increase in pH can result in precipitation of REEs as hydroxides or carbonates, exchange of REEs for H^+ on accessible mineral exchange sites, or adsorption of the REEs onto the surfaces of minerals (Humphris, 1984). Each of these processes reduces the dissolved REE concentration. Generally, REEs can be divided into 2 groups, the first of which is composed of more soluble elements (La-Gd), and the second group consists of the less soluble elements (Tb-Lu) (Sneller et al., 2000).

Speciation of REEs which determines solubility and bioavailability, depend on pH, salinity and the presence of anions (Sneller et al., 2000). As a consequence, speciation of REEs in salt water is different from fresh water. In salt water, between 70 and 96 % of the REEs is present as carbonate complexes, depending on the dissolved organic carbon (DOC) level, whereas in freshwater, humate complexes play a dominant role (Maas and Botterweg, 1993). At moderate to high pH values, complexation of REEs with carbonate species dominates and that heavy REEs (Tb-Lu) form stronger complexes with carbonate ions than light REEs due to an increase in stability constants with atomic number (Cantrell and Byrne, 1987; Millero, 1992; Lee and Bryne, 1993). In addition, heavy REEs are generally more sensitive to pH alternations than light members due to their lower basicity (Tu et al., 1994). With reference to Table 3 and Brookins (1989), in acidic to neutral pH, the REE^{3+} ions may be the dominant form. At very low pH, REE^{3+} and $(REE)SO_4^{+}$ are the most important. The halides are of minor importance even at very low pH. At neutral to slightly basic pH, the carbonate complexes become important with the light lanthanides (La-Gd) and one heavy lanthanide Tb, preferring $REE(CO_3)^{+}$. The other heavy lanthanides preferring $REE(CO_3)^{2-}$. As the pH approaches 10, hydrolysis products $REE(OH)_2^{+}$, $REE(OH)_3$(aq), and $REE(OH)_4^{-}$ become important. Unlike other REEs, behaviour of Ce and Eu is different, due to their occurrence of more than one redox-states (Ce^{3+}, Ce^{4+}; Eu^{2+}, Eu^{3+}). Under normal oxic circumstances, REEs are present in the trivalent oxidation states (Cotton, 1991), but Ce^{3+} is oxidized to Ce^{4+}, therefore forming CeO_2 which is insoluble in water. A different behaviour occurs for Eu in porewater in which Eu^{3+} is reduced to Eu^{2+}, resulting to increasing dissolved concentrations in porewater (Weltje et al., 2002b).

Potential Precipitates in Toxicity Tests

In natural systems lanthanides will possibly be undersaturated with respect to solid phases but at the higher concentrations often used for toxicity testing it is important to understand the potential for precipitation. It is well established that nominal and measured lanthanide concentrations do not necessarily match (see Test Methods section below) with measured values as much as a factor of two lower than nominal values. The likely solid phases in laboratory toxicity tests include hydroxide and/or carbonate phases. To investigate the likelihood of precipitation several equations were derived using National Institute of Standards and Technology (NIST) certified equilibrium constant values. The details of the equation derivations are given in Appendix A and the results of these calculations are shown for hydroxide in Figure 2 and for carbonate in Figure 3. Lanthanum hydroxide is most soluble at acidic pH and becomes less soluble as pH is increased. Alternatively, lanthanum carbonate goes through a solubility minimum around pH 8.2. The comparison acute toxicity values shown on Figure 2 demonstrate that for the measured La concentrations in toxicity tests (taken from Table 4) most studies were undersaturated with respect to $La(OH)_{3(s)}$. This is not the case for carbonate precipitation where most studies were actually performed in the regime where $La_2(CO_3)_{3(s)}$ is expected to precipitate. It should be noted that these calculations assumed a minimum amount of carbonate in that the only source of CO_2 was atmospheric. In general waters with even moderate alkalinity will have much greater carbonate concentrations and greater tendency to precipitate carbonate species. Thus, future toxicity testing on La elements should consider the possibility of carbonate precipitation and always utilize measured concentrations (see Test Methods below).

Geochemical Speciation

REEs have relatively high affinity to sediment (Sneller et al., 2000). Maas and Botterweg (1993) reported that log K_p (sm/w) of REEs in suspended matter and water in rivers is about 3 l kg^{-1}, where K_p is the partition or distribution coefficient. In oceans, this value is around 4, with the exception of Ce, for which the value is around 5 because of low solubility of the main form of Ce, CeO_2 in ocean, in which Ce is present as Ce^{4+}, as compared to trivalent state of other REEs. Therefore large amount of REEs are bound to sediment or suspended matter in the aquatic environment. In addition, log K_p (sediment to porewater) values for REEs are relatively higher compared to heavy metals such as Cu, Zn, Cd and Pb (log K_p sed/pw: 2.5 – 4 l kg^{-1}). In general, the variability in salinity, pH, composition of suspended matter, organic carbon content and Fe- and Mn-hydroxides, caused by the tidal movement, results in constantly changing equilibrium between sediment, pore water and surface water, and thus continuously changing REE concentrations in each partition (Bakkenist and Van De Wiel, 1995).

Bioavailability

Since most of lanthanides (99 %) are present in or bound to suspended matter and sediment, they are considered largely unavailable. Only a minor fraction is dissolved in sediment pore or surface water (Weltje, 2002). Among the dissolved species, bioavailability decreases in the following order: free ion, inorganic complex, and finally organic complex (Sun et al., 1997). Changes in ligand or ion concentration in the environment can change bioavailability of REEs, for example, uptake of Gd by the marine algae *Ulva lactua* was strongly reduced by increasing carbonate concentrations (Stanley and Byrne, 1990). Uptake of La by the freshwater algae *Scenedesmus pannonicus* was reduced at a lower pH, also at a higher Ca concentration (Demon et al., 1988; 1989). In addition, Marang et al. (2008) has shown that competitive interactions with H^+ inorganic species, and major cations (Ca^{2+}, Mg^{2+}) could influence Eu transport and bioavailability. Due to the tremendous use of lanthanides in industrial processes (see "Sources of Lanthanides" section), methylated lanthanides may be produced during the synthesis processes. For example, use of methylation to increase water solubility of lanthanum complex in optical industry (Spyroulias et al., 1998; Syproulias et al., 1998); cerium triflate as alternative for oxidizing aromatic hydrocarbons to synthesize useful organic chemicals (Molander, 1992). Methylmercury is known to have a better bioavailability than mercury, but biovailability of methylated lanthanides to aquatic organisms is not yet studied.

Although sediment-bound lanthanide is usually considered not to be available, the bioavailability may change when the equilibrium is disturbed. For instance, when sediment particles are ingested by organisms, the lower pH in digestive system of the organisms may shift the equilibrium, thus increases concentration of dissolved lanthanides. Sediment-rooting plants may also exude protons or chelating substances (e.g., malate, citrate) to increase availability of another trivalent metal, the essential but poorly soluble iron (Mori, 1999). Therefore sediment may become a source of bioavailable lanthanide in water. Lanthanides present in biota may also become available (trophic transfer) to other organisms feeding on them. By using sequential extraction, bioavailability of lanthanides from the sediment can be estimated. In the sediment, among the water-soluble, acid-extractable and organic / sulphide bound species, water soluble species of La, Gd, Sm, Ce, Y has the strongest correlation with the accumulation in the algae *Chlorella vulgaris* (Sun et al., 1998). It suggests that the REE released from the sediment is an important factor affecting the bioavailability to the algae.

Biological Effects on Aquatic Organisms

i). Acute Toxicity

Data from literature about acute and chronic toxicity of lanthanides to aquatic organisms are summarized in Table 4, 5 (freshwater) and Table 6 (saltwater). In general, most toxicity studies assessed effects of total/nominal lanthanides in the aquatic environments, fewer measured the dissolved concentrations, which is the most bioavailable form for biota. Due to the low solubility of lanthanides, it is not surprising that in freshwater (Table 4), the lower acute toxicity values (43 – 1232 μg REE $\cdot L^{-1}$) are found for studies measuring dissolved lanthanide concentration and the higher is for studies using nominal lanthanide concentrations (450 – 4,069,767 μg REE $\cdot L^{-1}$). There is an exception for the *Daphnia magna*, rainbow trout (*Oncorhynchus mykiss*) and the shrimp, *Thamnocephalus platyurus* which have extremely high toxicity values (> 44,000 μg REE $\cdot L^{-1}$) of dissolved lanthanides. Watson-Leung (2009)'s study on *D. magna* and rainbow trout tested on La leached from Phoslock®, with unclear water chemistry of the leachate other than hardness and pH, therefore it is difficult to explain for their resistance to La, compared to toxicity from salts. In general, among different species of cladocerans (*Daphnia magna*, *Daphnia carinata*, *Ceriodaphnia dubia*), increasing hardness from 22 to 210 mg $\cdot L^{-1}$ as $CaCO_3$ can reduce acute toxicity of lanthanides from 5 (total REEs) – 27 folds (dissolved REEs) (Table 4), and similar effect has been reported within *D. carinata* for La (Barry and Meehan, 2000). Freshwater algae, cladoceran and cnidarian have similar sensitivity to different REEs, with algae toxicity values from 450 – 4,400 μg REE $\cdot L^{-1}$; cladoceran toxicity values from 43.2 – 24,000 μg REE $\cdot L^{-1}$ and cnidarian toxicity values from 44 – 4,400 μg REE $\cdot L^{-1}$ in water of all hardness and all forms of REEs (dissolved / total). Toxicity of lanthanum to algae can be indirect associated with the removal of phosphate from the growth medium through the formation of $LaPO_4$ (Stauber, 2000). The zebrafish *Danio rerio* is ranked second for the sensitivity to lanthanides with acute toxicity values of total REEs from 19,000 – 25,000 in water hardness of 210 mg $\cdot L^{-1}$ as $CaCO_{3,}$ and sensitivity of them is comparable to the high ends of cladocerans in the same water hardness (Table 4). There is not sufficient information to compare toxicity among the fish species when Watson-Leung (2009) is not considered. The shrimp, *T. platyurus* is the most resistant to lanthanide. Since toxicity information is the most obtained for cladocerans, comparison is made for toxicity among REEs within them. Acute toxicity of total REEs to cladocerans in the freshwater environment (water hardness: 210 mg $\cdot L^{-1}$ as $CaCO_3$) is ranked as Nd > Gd > Sm > Pr ~ Dy > Ce > La (Table 4). Acute toxicity of REEs on sediment dwelling organisms is less studied. The EC_{50}s (movement inhibition) of different REEs on the oligochaete *Tubifex tubifex* were similar and they averaged to 78.1 mM (Filipi et al., 2007). This article was written in other language, water chemistry and form of lanthanide (total / dissolved) are unclear, therefore it is not included in Table 4.

There is scarce data on toxicity of lanthanides to saltwater organisms, with limited information on acute toxicity only (Table 6). Some of the data available is from a secondary source and written in another language, making the evaluation on toxicity to saltwater organisms difficult. In general, all REEs (light REEs vs heavy REEs; odd-numbered REEs vs even-numbered REEs) have about the same extent of toxicity to the marine algae *Skeletonema costatum* (nominal toxicity values: 4055 – 5009 μg REE $\cdot L^{-1}$) (Tai et al., 2010), but in the copepod, *Acartia tonsa*, toxicity of REEs is ranked as Ce > Sm > Gd > Nd > Pr > La > Dy and in the fish, *Poecilia reticulate*, toxicity is ranked as Pr > Nd > Sm > Gd > Ce > Dy > La (Table 6). Among the three saltwater species, copepod has the highest sensitivity to REEs, algae is the intermediate and the fish is the least sensitive.

Sneller et al. (2000) reported that, in general, acute toxicity of REEs is higher to marine organisms than freshwater organisms. From our literature data (Table 4, 6), algae in freshwater has a larger variation in responses (toxicity values: 450 – 15,470 μg total REE $\cdot L^{-1}$) to acute exposure of REEs than in saltwater (toxicity values: 4055 – 5009 μg total REE $\cdot L^{-1}$) which may be explained by the higher sensitivity of freshwater algae in water with low hardness. Saltwater crustacean (toxicity values: 150 – 3,600 μg total REE $\cdot L^{-1}$) are more susceptible to lanthanides than freshwater crustacean (toxicity values: 1,400 – 4,069,767 μg total REE $\cdot L^{-1}$) (Table 4, 6), although only one species of saltwater crustacean is compared. Data on different species of freshwater and saltwater fish are limited for evaluation.

ii). Chronic Toxicity

Chronic toxicity of lanthanides to freshwater organisms show similar pattern as the acute toxicity (Table 5). Increasing hardness reduces chronic toxicity to the cladocerans and a less conclusive effect is reported for the amphipods. Water hardness increasing from 40-48 mg$\cdot L^{-1}$ to 210 mg$\cdot L^{-1}$ as $CaCO_3$ almost reduces toxicity of total lanthanides to the cladocerans by 5 fold (Table 5). When water hardness increased from 18 – 124 mg$\cdot L^{-1}$ as $CaCO_3$, toxicity values increased from 0.01 – 191 μg REE $\cdot L^{-1}$ to 278 – 1665 μg REE $\cdot L^{-1}$ in *Hyallela azteca*. However, the latter range is a nominal value with no information on relative amount of dissolved REEs in the medium. Amphipod (toxicity values: 0.01 – 191 μg dissolved REE $\cdot L^{-1}$, hardness at 18 mg$\cdot L^{-1}$ as $CaCO_3$) and cladoceran (toxicity values: 8.7 – 842 μg dissolved REE$\cdot L^{-1}$, hardness at 85 – 160 mg$\cdot L^{-1}$ as $CaCO_3$) have the highest sensitivity, the midge *Chironomus dilutus* is the intermediate (NOEC of total REE > 880 μg$\cdot L^{-1}$, hardness at 138 – 179 mg$\cdot L^{-1}$ as $CaCO_3$) whereas the zebrafish *D. rerio* may have the lowest sensitivity (NOEC of total REEs ≥ 2,600 μg$\cdot L^{-1}$, hardness at 210 mg$\cdot L^{-1}$ as $CaCO_3$) (Table 5). Chronic toxicity (7 d LC_{50}) of each dissolved REE to *H. azteca* (Borgmann et al., 2005) is ranked as the follow: Tm > La > Nd > Lu > Ce > Pr > Sm > Tb > Yb > Eu > Ho > Gd > Dy > Er. Among all the chronic toxicity endpoints, brood size, length and weight of cladoceran neonates are more sensitive to lanthanides than survival and growth (Table 5). Main route of lanthanum uptake by daphnia may be through the carapace because Ca is constantly taken up during moult cycle to harden carapace, but La is absorbed and binds to the Ca binding sites (Das et al., 1988), thus interfere moulting cycle (Barry and Meehan, 2000).

There are also studies on microorganisms and physiological impacts of REEs on aquatic animals. Gd at nominal concentrations of 15.7 $mg \cdot L^{-1}$ and 47.2 $mg \cdot L^{-1}$ affected the population size of bacteria *E. coli* and protozoan *T. thermophila* respectively in a microcosm after they were exposed for from 7 d to over 100 d (Fuma et al., 2001). Sm, Er and Ho can increase the toxicity of sediment and sediment elutriate (certified reference material E7 sediment, National Water Research Institute, Burlington, ON) from 20 to over 4000-fold based on the microbial assays – Microtox and Luminotox solid phase assays (Blaise et al., 2008). Physiologically, Sm and Er caused cytotoxicity (activity of enzyme to reduce 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, test for cell viability and growth) of hepatocytes in rainbow trout (48 h threshold effect nominal concentrations: Sm > 43 $mg \cdot L^{-1}$; Er = 0.44 - 4.4 $mg \cdot L^{-1}$) (Blaise et al., 2008). Gd also caused cytotoxicity (nominal 24 h EC_{50} = 151.5 $mg \cdot L^{-1}$) and inhibited EROD activity (nominal 24 h EC_{50} = 58.4 $mg \cdot L^{-1}$) of primary rainbow trout hepatocytes (Laville et al., 2004). Glutamate-pyruvate transaminase activity in goldfish (*Carassius auratus*) liver was stimulated at 0.05 $mg \cdot L^{-1}$ Yb^{3+} and inhibited at higher concentrations. In addition, REEs affect antioxidant enzymatic activities in the fish. Activity of superoxide dismutase in the goldfish was stimulated at Yb higher than 0.05 $mg \cdot L^{-1}$ and catalase was strongly inhibited after 40 d of exposure, whereas glutathione S-transferase and glutathione peroxidise were stimulated at 0.05 $mg \cdot L^{-1}$ and inhibited at 0.1 $mg \cdot L^{-1}$ Yb (Guo et al., 2002).

Bioaccumulation by Aquatic Organisms

Lanthanides can be taken up by aquatic organisms from surface water, pore water and sediment. Bioaccumulation of lanthanides by aquatic organisms is summarized in Table 7 (freshwater) and 8 (saltwater). Among the freshwater bioaccumulation studies, only studies with sufficient data on BCF or both tissue and exposure concentrations are included in the tables. Note that, all the exposure concentrations of REEs in the freshwater studies are nominal values, therefore bioconcentration factor (BCF) may be under-estimated. Bioconcentration factor (BCF) in *Cyprinus carpio* is the highest in internal organs where is the major site for metabolism and detoxification, the intermediate is gill and the lowest is in muscle and skeleton (Tu et al., 1994;

Sun et al., 1996). However, BCF or tissue concentration of La in *C. carpio* in Sun et al. (1996) is much higher than in Tu et al. (1994) under an exposure at the same water hardness. It may be explained by the inverse relationship between exposure concentration and BCF (exposure concentration in Tu et al. 94 is higher than Sun et al. 96) (McGeer et al., 2003). In general, bioaccumulation by the duckweed is higher than the fish, *C. carpio* (Table 7). As reported by Weltje et al. (2002b), BCF of REEs (La – Lu) in freshwater plants and mollusc is between 10,000 and 100,000 $L \cdot kg^{-1}$ dry weight (duckweed *Lemna minor*: 10,000; pondweed *Potamogeton pectinatus*: 5,000 – 300,000; snail soft tissue: 5,000 – 200,000; bivalve soft tissue: 3,000 -30,000). There was only a low extent of biomagnifications (biomagnification factor: 5.5) from plant (food) to snail tissue due to a similarity between BCF of pondweed and snail tissue (Weltje et al., 2002b).

In salt water, most of the studies only measured tissue concentration of organisms collected from the field, but no REE concentration or salinity were reported (Table 8). There is no significant difference in tissue concentration of REEs among the cephalopod, mussel and scallop from the unpolluted sites (Lobel et al., 1991; Riget et al., 1996; Bustamante and Miramand, 2005; Pernice et al., 2009), but there is a variation of distribution of REEs among different tissues in the body. When the sites are polluted by REEs, invertebrates tend to distribute most of REEs in the digestive gland, following the gill, gonad and kidney, and the least is in the muscle (Table 8). There was 61 - 79% Ce, 72 - 81%, La, 55 - 75% Nd in the digestive glands of the two *Nautilus* species (Pernice et al., 2009). It highlighted that digestive gland is the important site for bioaccumulation of REEs. Stronkhorst and Yland (1998) also reported the BCF of an estuarine amphipod, *Corophium volutator* decreases with increasing atomic number of REEs (La: 28,840; Ce: 48,978; Pr: 38,905; Nd: 29,512; Sm: 17,783; Eu: 11,220; Gd: 13,183; Tb: 12,882; Dy: 9,550; Ho: 8,511; Er: 7,413; Tm: 6,310; Yb: 6,607; Lu: 4,786; all in $l\ kg^{-1}$). Since this data set is from a secondary reference written in other language, and information is lacking such as BCF unit expression (wet or dry weight), it is not included in Table 7 or 8. However, a trend of higher BCF is observed in the amphipod than the freshwater fish, *C. carpio*.

Data Gaps & Recommendations for Toxicity Testing

i). Test Elements

Lighter REEs (La – Gd) may have a higher priority than heavy lanthanides (Tb – Lu) for toxicity testing because they are naturally more abundant. In addition, they are commonly applied in industries, e.g., La (fertilizers); Ce, Sm (fuel additive, nanoparticles); Gd (pharmaceutical products) (Barry and Meehan, 2000; Laville et al., 2004; Blaise et al., 2008; Gaiser et al., 2009). Ligher REEs also have a higher solubility which suggests they are more bioavailable to biota. Among the lighter REEs, Ce and Eu need to be particularly concerned because they have different physicochemical behaviour from other REEs. Pm can be neglected for testing due to the absence of their natural occurrence, except for studying effects of their radioisotopes.

ii). Test Systems

Freshwater environment is relatively more studied than saltwater environment, with both acute and chronic data available for fresh water, but only acute data available for salt water. Sneller et al. (2000) reported that saltwater organisms in general are more susceptible to lanthanides than freshwater organisms. Our literature also demonstrates this trend in the crustaceans (Table 4 & 6), otherwise data is limited from a few species of each system to support this statement. Toxicity of lanthanides in pore water which has a lower pH, thus a higher dissolved concentration, and the sediment which is the largest reservoir for bioavailable lanthanides in water are rarely studied (only Borgmann et al. 2005; Watson-Leung, 2009, Table 5). Although sediment bound lanthanides are considered to be not bioavailable, bioavailability may change when the equilibrium is disturbed (e.g., change in pH) in the sediment and biota systems. In general, data is still lacking on different areas (see sections below) of each aquatic system.

iii). Test Species

A lot of tests have been conducted on the cladocerans, but tests on other species from different families are insufficient. US EPA (Stephan et al., 1985; EPA, 1994) requires toxicity data from a suite of aquatic organisms for developing the water quality criteria and conducting the ecological risk assessment. They are algae; fish (salmonid & non-salmonid); planktonic crustacean; benthic crustacean; insect and annelid or mollusk. Among the toxicity tests from the literature, we are lack of studies on the freshwater salmonids e.g., rainbow trout (only Watson-Leung, 2009, Table 4) and sensitive non-salmonid species. Fathead minnow is a recommended sensitive non-salmonid species for toxicity testing on their life cycle embryo-larval/teratogenicity (chronic) by EPA (1994). There are also insufficient data on the freshwater amphipod (only Borgmann et al., 2005, Table 5), different REEs on freshwater insect (only La in Watson-Leung, 2009), annelid worms and mollusks. The oligochaete *Lumbriculus* or *Tubifex tubifex* is recommended to be the tested freshwater annelid because toxicity tests have been well developed for them (Chapman et al., 1999), whereas the pond snail *Lymnaea stagnalis* as the tested freshwater mollusk as it is very sensitive to pollutants (e.g., Grosell et al., 2006). Very limited toxicity data is available for acute tests on saltwater organisms (one species of algae, fish and invertebrates only, Table 6) and chronic toxicity tests on all saltwater species are lacking. Suggested chronic tests on saltwater organisms are survival and development of marine bivalve larvae, sea urchin fertilization, mysid survival, growth and fecundity which are all standard toxicity tests of EPA (1994).

iv). Test Methods

About 50 % of toxicity values from literature are nominal or total REEs concentrations. However, dissolved lanthanide concentrations can deviate from nominal values of more than 50 % (Lürling and Tolman, 2010) since a lot of them can be adsorbed to containers or precipitate within 24 h of exposure (Barry and Meehan, 2000). Since only dissolved lanthanide can cause toxicity to the biota, it is important to measure concentrations in dissolved form. Keep the containers for equilibrium with lanthanides for 24 h before exposure starts, and measure the dissolved concentrations regularly during the toxicity tests. Daily renewal of the medium or flow-through system is also important to maintain the lanthanide concentrations. Using nominal or total lanthanide concentration for calculating BCF is also a concern in bioaccumulation studies because the ratio of lanthanides taken up from the water is under-estimated.

v). Test Topics

Water chemistry has been demonstrated to play an important role on metal toxicity. Some abiotic ligands (Ca; Mg; carbonates; pH) have already been shown to affect uptake of lanthanides by algae, therefore effects of a wide range of toxicity modifying factors (Ca, Mg, Na, pH, carbonates, hydroxides, DOC, salinity) are of importance for further investigations especially in the fish and invertebrates. In order to fulfill the goals, water chemistry parameters need to be measured throughout the toxicity tests. In freshwater system, since DOC plays an important role on speciation of lanthanides (Maas and Botterweg, 1993), quality and quantity of dissolved organic matter on toxicity and bioavailability of lanthanides are of interest for investigations. Biotic ligand e.g., gill in fish, is the site of toxic action, similar to metals, physiological binding constants of lanthanides (Log K, B_{max}) can be quantified on the fish gill or whole body of invertebrates (Di Toro et al., 2001). In addition, short-term lanthanide burden (3-24 h) can be measured to determine if there is a relationship between short-term burden and acute or chronic toxicity on the organisms. These binding constants do not exist for lanthanides. Collection of all these data will help to develop a new model, or add data to an existing model e.g., Biotic Ligand Model for predicting toxicity of lanthanides, as well as metals. Toxicity of lanthanides in different form, e.g., methylated or organic lanthanides should not be neglected for testing as bioavailability of them compared to from salts is less known. In addition, as dietary toxicity of pollutants, e.g., metals, are getting more interests, effects of lanthanide from diet through trophic transfer is not studied yet.

Table 1: Search strategies, keywords & results

Keywords		Results	
		# of papers / abstracts screened ("hits")	# papers retained for further screening
Rare earth elements	environmental toxicity	1464	20
	in fish	135	10
	bioavailability	59	3
Lanthanides	toxicity	614	20
	environment toxicity	578	67
	bioavailability	11	3
Lanthanum	ecotoxicity	0	0
	environmental toxicity	28	15
	speciation	14	2
	bioaccumulation	6	6
	in fish	79	16
	in invertebrates	294	35
	bioavailability	24	2
Cerium	ecotoxicity	0	0
	environmental toxicity	17	14
	toxicity	221	126
	speciation	9	7
	bioaccumulation	7	7
	physiology	134	0
	in fish	138	20
	in invertebrates	37	14
	in plants	249	36
	bioavailability	17	1
Praseodymium	ecotoxicity	0	0
	environmental toxicity	0	0
	toxicity	19	13
	speciation	0	0
	bioaccumulation	0	0
	in fish	1	1
	in invertebrates	2	0
	in plants	8	5
	bioavailability	4	0
Neodymium	ecotoxicity	0	0
	environmental toxicity	0	0

	toxicity	31	14
	speciation	1	0
	bioaccumulation	0	0
	in fish	1	1
	in invertebrates	0	0
	in plants	17	8
	bioavailability	6	0
Promethium	ecotoxicity	0	0
	environmental toxicity	0	0
	toxicity	9	7
	speciation	0	0
	bioaccumulation	0	0
	in fish	0	0
	in invertebrates	0	0
	in plants	4	1
	bioavailability	1	0
Samarium	ecotoxicity	1	1
	environmental toxicity	3	1
	toxicity	85	18
	speciation	0	0
	bioaccumulation	0	0
	in fish	1	1
	in invertebrates	9	3
	in plants	11	9
	bioavailability	10	0
Europium	ecotoxicity	0	0
	environmental toxicity	0	0
	toxicity	26	25
	physiology	812	104
	speciation	24	12
	bioaccumulation	9	9
	in fish	19	3
	in invertebrates	23	9
	in plants	40	17
	bioavailability	7	0
Gadolinium	ecotoxicity	0	0
	environmental toxicity	35	9
	toxicity	519	32

	speciation	13	2
	bioaccumulation	1	1
	in fish	28	7
	in invertebrates	52	6
	in plants	74	3
	bioavailability	10	0
Terbium	ecotoxicity	0	0
	environmental toxicity	6	5
	toxicity	19	10
	speciation	0	0
	bioaccumulation	0	0
	in fish	29	3
	in invertebrates	16	0
	in plants	14	7
	bioavailability	4	0
Dysprosium	ecotoxicity	0	0
	environmental toxicity	2	1
	toxicity	16	12
	speciation	0	0
	bioaccumulation	0	0
	in fish	0	0
	in invertebrates	1	1
	in plants	18	2
	bioavailability	5	0
Holmium	ecotoxicity	0	0
	environmental toxicity	2	2
	toxicity	21	9
	speciation	0	0
	bioaccumulation	0	0
	in fish	1	1
	in invertebrates	1	1
	in plants	3	3
	bioavailability	2	0
Erbium	ecotoxicity	1	1
	environmental toxicity	1	1
	toxicity	9	4
	speciation	0	0
	bioaccumulation	0	0

	in fish	1	1
	in invertebrates	4	3
	in plants	6	2
	bioavailability	1	0
Thulium	ecotoxicity	0	0
	environmental toxicity	0	0
	toxicity	4	3
	speciation	1	1
	bioaccumulation	0	0
	in fish	0	0
	in invertebrates	0	0
	in plants	4	2
	bioavailability	2	0
Ytterbium	ecotoxicity	0	0
	environmental toxicity	2	1
	toxicity	22	10
	speciation	1	1
	bioaccumulation	0	0
	in fish	10	7
	in invertebrates	1	1
	in plants	22	3
	bioavailability	2	0
Lutetium	ecotoxicity	0	0
	environmental toxicity	0	0
	toxicity	42	11
	speciation	4	3
	bioaccumulation	1	1
	in fish	0	0
	in invertebrates	1	1
	in plants	3	2
	bioavailability	3	0
Total		6359	862
# papers retained for detailed review (excluding duplicates)			577
# additional articles from literature			52
Total papers entered into EndNote database for review			**629**

Note: "Bioavailability" search was only run in Web of Science database and only articles relevant to aquatic organisms were retained.

Table 2. Rank of abundance of lanthanides in earth crust and their associated minerals

Lanthanide	Abundance in Earth's crust (rank)	Mineral where found
Lanthanum (La)	28	monazite; bastnasite
Cerium (Ce)	26	monazite; bastnasite; allanite.
Praseodymium (Pr)	37	monazite; bastnasite
Neodymium (Nd)	27	monazite; bastnasite
Samarium (Sm)	40	monazite, bastnasite, samarskite
Europium (Eu)	50	monazite, bastnaesite
Gadolinium (Gd)	41	samarskite, gadolinite, monazite,some varieties of Norwegian ytterspar
Terbium (Tb)	58	cerite, gadolinite, monazite, xenotime, euxenite
Dysprosium (Dy)	42	xenotime, fergusonite, gadolinite, euxenite, polycrase, blomstrandine, monazite, bastnäsite
Holmium (Ho)	55	gadolinite, monazite
Erbium (Er)	43	monazite
Thulium (Tm)	61	euxenite, gadolinite, blomstrandine
Ytterbium (Yb)	29	monazite, euxenite, xenotime.
Lutetium (Lu)	59	gadolinite, monazite, and xenotime

*Promethium is a by-product from decay of uranium and it is not naturally present in the earth crust.

Table 3. A list of dominant species of lanthanide complex in solution (Moeller and Vincenti, 1965; Cantrell and Byrne, 1987; Brookins, 1989).

Inorganic	**Remarks**
$REE(X)^{2+}$	X= F, Cl, Br, I
$REE(X_2)^{+}$	X= Cl, Br
$REE(ClO_4)^{2+}$	
$REE(NO_3)^{2+}$	
$REE(P_2O_7)_n^{(3-4n)+}$	n=1, 2
$REE(SO_4)^{+}$	
$REE(SO_4)^{2-}$	
$REE(CO_3)^{+}$	
$REE(CO_3)^{2-}$	
Organic	
$REE(C_2H_3O_2)_n^{(3-n)+}$	n= 1-3
$REE(HOCH_2COO)_n^{(3-n)+}$	n= 1-4
$REE(EDTA)^{-}$	
$REE(NTA)_n^{(3-n)+}$	n= 1, 2
$REE(HEDTA)(IMDA)^{2-}$	
$REE(HEDTA)(OH)^{-}$	
$REE(b\text{-}diketon)_n^{(3-n)+}$	n= 1-3
$REE(PDC)_n^{(3-2n)+}$	n=1-3
$REE(NO_3)_3 \cdot 3TBP$	

Table 4. Acute toxicity of lanthanides to freshwater organisms

Organism	Species	Base element	Compound	Organism age or Size	Exposure method*	Element conc.*	Hardness (mg $CaCO_3$ l^{-1})	pH	Temp (°C)	Endpoint	Effect	Toxicity value (µg REE l^{-1})	Reference*
Algae	*Pseudokirchneriella subcapitata*	Sm	Sm_2O_3	--	S	D[a]	--	6-6.8	--	72 h IC25	Growth	430 – 4,300[b]	(Blaise et al., 2008)
Algae	*Pseudokirchneriella subcapitata*	Er	Er_2O_3	--	S	D[a]	--	6-6.8	--	72 h IC25	Growth	440 – 4,400[b]	(Blaise et al., 2008)
Algae	*Pseudokirchneriella subcapitata*	Ho	Ho_2O_3	--	S	D[a]	--	6-6.8	--	72 h IC25	Growth	440 – 4,400[b]	(Blaise et al., 2008)
Algae	*Pseudokirchneriella subcapitata*	Ce	CeO_2 nanoparticles	~10^4 cells ml^{-1}	S	N, T	24.2	7.4	25	72 h IC50	Growth	8,302 - 15,470[c]	(Van Hoecke et al., 2009)
Algae	*Selenastrum capricornutum*	La	$LaCl_3$	--	S	N, T	<10	--	24	72 h IC50	Growth	450	(NICNAS, 2001)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	neonate	SR	M, D	98	7.8	20	48 h EC50	Survival	49	(Barry and Meehan, 2000)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	neonate	SR	M, D	160	7.5	20	24 h EC50	Survival	1232.4	(Barry and Meehan, 2000)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	neonate	SR	M, D	160	7.5	20	48 h EC50	Survival	1180	(Barry and Meehan, 2000)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	neonate	SR	M, D	22	7.5	20	24 h EC50	Survival	484.5	(Barry and Meehan, 2000)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	neonate	SR	M, D	22	7.5	20	48 h EC50	Survival	43.2	(Barry and Meehan, 2000)
Cladoceran	*Daphnia magna*	Ce	CeO_2 nano & bulk particles	neonate	S	N, T	84.9	--	--	96 h LC50	Survival	NOEC: 8139.5	(Gaiser et al., 2009)
Cladoceran	*Daphnia magna*	Ce	CeO_2 nanoparticles	<24 h	S	N, T	249	7.4	20	48 h EC50	Survival	NOEC: 813953	(Van Hoecke et al., 2009)
Cladoceran	*Daphnia magna*	La	--	<24 h	S	M, T	210	6.5-8.2	--	48 h EC50	Survival	24,000	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Ce	--	<24 h	S	M, T	210	6.2-8.0	--	48 h EC50	Survival	22,000	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Pr	--	<24 h	S	M, T	210	6.2-8.2	--	48 h EC50	Survival	9,000	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Nd	--	<24 h	S	M, T	210	6.4-8.3	--	48 h EC50	Survival	1,400	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Sm	--	<24 h	S	M, T	210	6.3-8.2	--	48 h EC50	Survival	7,600	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Gd	--	<24 h	S	M, T	210	6.5-8.2	--	48 h EC50	Survival	6,800	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Dy	--	<24 h	S	M, T	210	6.5-8.1	--	48 h EC50	Survival	9,100	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	La	[d]Phoslock®	<24 h	S	M, D	192	6.8-8.3	21	48 h LC50	Survival	NOEC > 63270	(Watson-Leung, 2009)
Cladoceran	*Ceriodaphnia dubia*	La	[d]Phoslock®	<24 h	S	M, D	84.9	7	25	48 h EC50	Survival	80	(Stauber, 2000)
Cladoceran	*Ceriodaphnia dubia*	La	$LaCl_3$	<24 h	S	M, T	40-48	7	25	48 h EC50	Survival	5,000	(NICNAS, 2001)

Table 4. Acute toxicity of lanthanides to freshwater organisms (con't)

Organism	Species	Base element	Compound	Organism age or Size	Exposure method*	Element conc.*	Hardness (mg $CaCO_3$ l^{-1})	pH	Temp (°C)	Endpoint	Effect	Toxicity value (µg REE l^{-1})	Reference*
Cnidarian	*Hydra attenuata*	Sm	Sm_2O_3	adult	S	D[a]	130	6-6.8	20 – 24	96 h EC50	Morphology	430 – 4,300[b]	(Blaise et al., 2008)
Cnidarian	*Hydra attenuata*	Er	Er_2O_3	adult	S	D[a]	130	6-6.8	20 – 24	96 h EC50	Morphology	440 – 4,400[b]	(Blaise et al., 2008)
Cnidarian	*Hydra attenuata*	Ho	Ho_2O_3	adult	S	D[a]	130	6-6.8	20 – 24	96 h EC50	Morphology	44 – 440[b]	(Blaise et al., 2008)
Shrimp	*Thamnocephalus platyurus*	Sm	Sm_2O_3	20-22 h instar (stage II-III)	S	D[a]	--	6-6.8	25	24 h LC50	Survival	> 43,000[b]	(Blaise et al., 2008)
Shrimp	*Thamnocephalus platyurus*	Er	Er_2O_3	20-22 h instar (stage II-III)	S	D[a]	--	6-6.8	25	24 h LC50	Survival	> 44,000[b]	(Blaise et al., 2008)
Shrimp	*Thamnocephalus platyurus*	Ho	Ho_2O_3	20-22 h instar (stage II-III)	S	D[a]	--	6-6.8	25	24 h LC50	Survival	> 44,000[b]	(Blaise et al., 2008)
Shrimp	*Thamnocephalus platyurus*	Ce	CeO_2 nanoparticles	20-22 h instar (stage II-III)	S	N, T	--	7.4	25	24 h LC50	Survival	4,069,767	(Van Hoecke et al., 2009)
Fish	*Melanotaenia duboulayi*	La	[d]Phoslock®	juvenile	S	M, D	84.9	6.9	--	96 h EC50	Immobilization	NOEC: 127	(Stauber, 2000)
Fish	*Melanotaenia duboulayi*	La	$LaCl_3$	juvenile	S	M, D	40-48	6.5–8.1	23.4 – 24.5	96 h EC50	Immobilization	NOEC < 600	(NICNAS, 2001)
Fish	*Danio rerio*	Ce	CeO_2 nanoparticles	<30 min after spawning	S	N, T	209	7.4	28	72 h EC10	Hatching	NOEC: 162791	(Van Hoecke et al., 2009)
Fish	*Danio rerio*	La	--	--	SR	M, T	210	6.4-8.2	--	96 h LC50	Survival	23,000	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Ce	--	--	SR	M, T	210	6.3-8.1	--	96 h LC50	Survival	22,000	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Pr	--	--	SR	M, T	210	6.2-8.2	--	96 h LC50	Survival	25,000	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Nd	--	--	SR	M, T	210	6.5-8.4	--	96 h LC50	Survival	21,000	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Sm	--	--	SR	M, T	210	6.3-8.0	--	96 h LC50	Survival	22,000	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Gd	--	--	SR	M, T	210	6.5-8.0	--	96 h LC50	Survival	19,000	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Dy	--	--	SR	M, T	210	6.2-8.0	--	96 h LC50	Survival	25,000	(Den-Ouden, 1995)[SD]
Fish	*Oncorhynchus mykiss*	La	[d]Phoslock®	juvenile	S	M, D	128	7.1-8.4	15	96 h LC50	Survival	NOEC > 63270	(Watson-Leung, 2009)

*S = static; SR = static-renewal; M = measured; N = nominal; D = dissolved (pass through ≤ 0.45 µm filter); T = total; SD = secondary data, whereas unmarked references are primary data

[a]Dissolved lanthanide concentration is calculated from passing the exposure medium through a 0.22 mm preweighed filter to get the insoluble concentration (Blaise et al. 2008)

[b]Exact endpoint values are not reported in the article, instead a range is reported according to the EU Directive 93/67/EEC

[c]EC50 range is given for nanoparticles with different sizes (14, 20, 29 nm) (Van Hoecke et al. 2009)

[d]Phoslock® is a lanthanum modified clay for water treatment

Table 5. Chronic toxicity of lanthanides to freshwater organisms

Organism	Species	Base element	Compound	Element conc.*	Hardness (mg $CaCO_3$ l^{-1})	pH	Temp (°C)	Endpoint	Effect	Toxicity value (µg REE l^{-1})	Reference*
Duckweed	*Lemna minor*	La	$LaCl_3$	N, T	54.8	5.1	25	9 d EC50	Growth	NOEC: 1.39	(Weltje et al., 2002a)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	M, D	160	7.5	20	Life cycle LC50	Survival	LOEC: 39	(Barry and Meehan, 2000)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	M, D	160	7.5	20	Life cycle EC50	Age at maturity	LOEC: 39	(Barry and Meehan, 2000)
Cladoceran	*Daphnia carinata*	La	$LaCl_3$	M, D	160	7.5	20	Life cycle EC50	Brood size	LOEC: 30	(Barry and Meehan, 2000)
Cladoceran	*Daphnia magna*	La	[a]Phoslock®	M, D	88	7.6	20	5 d EC50	Weight	8.7[b]	(Lürling and Tolman, 2010)
Cladoceran	*Daphnia magna*	La	[a]Phoslock®	M, D	88	7.6	20	5 d EC50	Length	15.6[b]	(Lürling and Tolman, 2010)
Cladoceran	*Daphnia magna*	La	$La(NO_3)_3$	M, D	88	7.6	20	Life cycle LC50	Survival	NOEC = 1,001	(Lürling and Tolman, 2010)
Cladoceran	*Daphnia magna*	La	$La(NO_3)_3$	M, D	88	7.6	20	Life cycle EC50	Brood size	NOEC = 1,001	(Lürling and Tolman, 2010)
Cladoceran	*Daphnia magna*	La	$La(NO_3)_3$	M, D	88	7.6	20	Life cycle EC50	Growth	NOEC = 1,001	(Lürling and Tolman, 2010)
Cladoceran	*Daphnia magna*	Ce	CeO_2 nanoparticles	N, T	249	7.4	20	21 d LC50	Survival	30035 – 57872[c]	(Van Hoecke et al., 2009)
Cladoceran	*Daphnia magna*	Ce	CeO_2 nanoparticles	N, T	249	7.4	20	21 d EC50	Brood size	16686 – 34756[c]	(Van Hoecke et al., 2009)
Cladoceran	*Daphnia magna*	Nd	--	M, T	210	7.6-8.7	--	21 d LC50	Survival	NOEC: 1,600	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Nd	--	M, T	210	7.6-8.7	--	21 d EC50	Fitness	NOEC: 1,600	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Dy	--	M, T	210	–	--	21 d EC50	Reproduction[d]	NOEC < 200	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Dy	--	M, T	210	7.9-8.5	--	21 d LC50	Survival	NOEC > 2,100	(Den-Ouden, 1995)[SD]
Cladoceran	*Daphnia magna*	Dy	--	M, T	210	7.9-8.5	--	21 d EC50	Fitness	NOEC > 2,100	(Den-Ouden, 1995)[SD]
Cladoceran	*Ceriodaphnia dubia*	La	[a]Phoslock®	M, D	84.9	7.9	25	7 d LC50	Survival	842	(NICNAS, 2001)
Cladoceran	*Ceriodaphnia dubia*	La	[a]Phoslock®	M, D	84.9	7.9	25	7 d EC50	Brood size	154	(NICNAS, 2001)
Cladoceran	*Ceriodaphnia dubia*	La	$LaCl_3$	M, T	40-48	7.9	25	7 d LC50	Survival	510	(Borgmann et al., 2005)
Cladoceran	*Ceriodaphnia dubia*	La	$LaCl_3$	M, T	40-48	7.9	25	7 d EC50	Brood size	430	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Ce	CeO_2	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	651	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Ce	CeO_2	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	32	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Dy	Dy_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	897	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Dy	Dy_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	162	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Er	Er_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	929	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Er	Er_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	191	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Eu	Eu_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	717	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Eu	Eu_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	112	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Gd	Gd_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	599	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Gd	Gd_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	150	(Borgmann et al., 2005)

Table 5. Chronic toxicity of lanthanides to freshwater organisms (con't)

Organism	Species	Base element	Compound	Element conc.*	Hardness (mg $CaCO_3$ l^{-1})	pH	Temp (°C)	Endpoint	Effect	Toxicity value (µg REE l^{-1})	Reference*
Amphipod	*Hyallela azteca*	Ho	Ho_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	755	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Ho	Ho_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	143	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	La	La_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	1665	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	La	La_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	18	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Lu	Lu_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	1054	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Lu	Lu_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	29	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Nd	Nd_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	511	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Nd	Nd_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	55	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Pr	Pr_6O_{11}	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	441	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Pr	Pr_6O_{11}	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	35	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Sm	Sm_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	846	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Sm	Sm_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	74	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Tb	Tb_4O_7	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	693	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Tb	Tb_4O_7	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	84	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Tm	Tm_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	739	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Tm	Tm_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	0.01	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Yb	Yb_2O_3	N, T	124	7.2-9.0	24 - 25	7 d LC50	Survival	278	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	Yb	Yb_2O_3	M, D	18	6.4-8.7	24 - 25	7 d LC50	Survival	69	(Borgmann et al., 2005)
Amphipod	*Hyallela azteca*	La	[a]Phoslock®	M, D	145-208	8.0-8.6	23	14 d	Survival	NOEC: 7	(Watson-Leung, 2009)
Amphipod	*Hyallela azteca*	La	[a]Phoslock®	M, D	145-208	8.0-8.6	23	14 d	Growth	NOEC: 7	(Watson-Leung, 2009)
Insect	*Chironomus dilutus*	La	[a]Phoslock®	M, T	138-179	8.1-9.1	21-23	10 d	Survival	NOEC: 880	(Watson-Leung, 2009)
Insect	*Chironomus dilutus*	La	[a]Phoslock®	M, T	138-179	8.1-9.1	21-23	10 d	Weight	NOEC: 880	(Watson-Leung, 2009)
Insect	*Hexagenia spp.*	La	[a]Phoslock®	M, D	101-125	8.1-8.5	22	21 d	Survival	NOEC < 3	(Watson-Leung, 2009)
Insect	*Hexagenia spp.*	La	[a]Phoslock®	M, D	101-125	8.1-8.5	22	21 d	Weight	NOEC < 3	(Watson-Leung, 2009)
Fish	*Danio rerio*	Dy	--	M, T	210	--	--	30 d EC50	Weight	NOEC: 3,000	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Dy	--	M, T	210	6.7-8.4	--	30 d LC50	Survival	NOEC: 2,600	(Den-Ouden, 1995)[SD]
Fish	*Danio rerio*	Dy	--	M, T	210	6.7-8.4	--	30 d EC50	Fitness	NOEC: 3,800	(Den-Ouden, 1995)[SD]

*M = measured; N = nominal; D = dissolved (pass through ≤ 0.45 µm filter), T = total; SD = secondary data, whereas unmarked references are primary data
[a]Phoslock® is a lanthanum modified clay for water treatment
[b]EC50 of La is calculated from the measured percentage of La (0.001 %) leached from Phoslock® (Lürling and Tolman, 2010)
[c]EC50 range is given for nanoparticles with different sizes (14, 20, 29 nm) (Van Hoecke et al. 2009)
[d]No information is given for what reproduction endpoint the study assessed.

Table 6. Acute toxicity of lanthanides to saltwater organisms

Organism	Species	Base element	Compound	Organism age or Size	Exposure method*	Element conc.*	Salinity (‰)	Temp (ºC)	Endpoint	Effect	Toxicity value (µg REE l^{-1})	Reference*
Algae	*Skeletonema costatum*	La	$LaCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4054.5	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Ce	$Ce(NO_3)_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4155.2	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Nd	$NdCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4375	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Sm	$SmCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4313.5	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Eu	$Eu(NO_3)_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4432.3	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Gd	$Gd(NO_3)_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4686	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Tb	$TbCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4536.3	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Dy	$DyCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4593.9	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Ho	$HoCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4829.9	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Er	$Er(NO_3)_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4962.1	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Tm	$TmCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4866	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Yb	$YbCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	4935.7	(Tai et al., 2010)
Algae	*Skeletonema costatum*	Lu	$LuCl_3$	10^4 cells ml^{-1}	S	N, T	32–35	25	72 h EC50	Growth	5008.5	(Tai et al., 2010)
Copepod	*Acartia tonsa*	Ce	–	6-8 d	S	–	28	–	48 h LC50	Survival	150	(Bowmer et al., 1992)[SD]
Copepod	*Acartia tonsa*	Dy	–	6-8 d	S	–	28	–	48 h LC50	Survival	3600	(Bowmer et al., 1992)[SD]
Copepod	*Acartia tonsa*	Gd	–	6-8 d	S	–	28	–	48 h LC50	Survival	520	(Bowmer et al., 1992)[SD]
Copepod	*Acartia tonsa*	La	–	6-8 d	S	–	28	–	48 h LC50	Survival	1040	(Bowmer et al., 1992)[SD]
Copepod	*Acartia tonsa*	Nd	–	6-8 d	S	–	28	–	48 h LC50	Survival	850	(Bowmer et al., 1992)[SD]
Copepod	*Acartia tonsa*	Pr	–	6-8 d	S	–	28	–	48 h LC50	Survival	920	(Bowmer et al., 1992)[SD]
Copepod	*Acartia tonsa*	Sm	–	6-8 d	S	–	28	–	48 h LC50	Survival	420	(Bowmer et al., 1992)[SD]
Fish	*Poecilia reticulata*	Ce	–	0.15 g, 1.9 cm	SR	–	28	21-25	96 h LC50	Survival	11200	(Hooftman et al., 1992)[SD]
Fish	*Poecilia reticulata*	Dy	–	0.15 g, 1.9 cm	SR	–	28	21-25	96 h LC50	Survival	15400	(Hooftman et al., 1992)[SD]
Fish	*Poecilia reticulata*	Gd	–	0.15 g, 1.9 cm	SR	–	28	21-25	96 h LC50	Survival	10800	(Hooftman et al., 1992)[SD]
Fish	*Poecilia reticulata*	La	–	0.15 g, 1.9 cm	SR	–	28	21-25	96 h LC50	Survival	47000	(Hooftman et al., 1992)[SD]
Fish	*Poecilia reticulata*	Nd	–	0.15 g, 1.9 cm	SR	–	28	21-25	96 h LC50	Survival	9600	(Hooftman et al., 1992)[SD]
Fish	*Poecilia reticulata*	Pr	–	0.15 g, 1.9 cm	SR	–	28	21-25	96 h LC50	Survival	4500	(Hooftman et al., 1992)[SD]
Fish	*Poecilia reticulata*	Sm	–	0.15 g, 1.9 cm	SR	–	28	21-25	96 h LC50	Survival	10600	(Hooftman et al., 1992)[SD]

*S = static; SR = static-renewal; N = nominal; T = total; SD = secondary data, whereas unmarked references are primary data

Table 7. Bioaccumulation of lanthanides by freshwater organisms

Organism	Species	Base element	Compound	Hardness (mg $CaCO_3$ l^{-1})	Duration	Tissue	pH	Exposure conc.[a] (µg l^{-1})	Tissue conc.[b] (µg REE g^{-1})	BCF[c] (L kg^{-1})	Reference*
Duckweed	*Lemna minor*	La	$LaCl_3$	54.8	48 h	Whole plant	5.1-5.6	1.39	1.7	826.3[d]	(Weltje et al., 2002a)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	45 d	Skeleton	6	500	2.8	5.6	(Tu et al., 1994)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	45 d	Muscle	6	500	1.3	2.6	(Tu et al., 1994)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	45 d	Gill	6	500	7	13.9	(Tu et al., 1994)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	45 d	Internal organs	6	500	38.9	77.8	(Tu et al., 1994)
Fish	*Cyprinus carpio*	Gd	$Gd(NO_3)_3$	53-60	45 d	Skeleton	6	500	2.3	4.6	(Tu et al., 1994)
Fish	*Cyprinus carpio*	Gd	$Gd(NO_3)_3$	53-60	45 d	Muscle	6	500	1.6	3.2	(Tu et al., 1994)
Fish	*Cyprinus carpio*	Gd	$Gd(NO_3)_3$	53-60	45 d	Gill	6	500	5.3	10.7	(Tu et al., 1994)
Fish	*Cyprinus carpio*	Gd	$Gd(NO_3)_3$	53-60	45 d	Internal organs	6	500	42.3	84.6	(Tu et al., 1994)
Fish	*Cyprinus carpio*	Ce	$Ce(NO_3)_3$	53-60	43 d	Muscle	6	270	0.05	0.2	(Sun et al., 1996)
Fish	*Cyprinus carpio*	Ce	$Ce(NO_3)_3$	53-60	43 d	Skeleton	6	270	1.6	5.9	(Sun et al., 1996)
Fish	*Cyprinus carpio*	Ce	$Ce(NO_3)_3$	53-60	43 d	Gill	6	270	3.5	12.8	(Sun et al., 1996)
Fish	*Cyprinus carpio*	Ce	$Ce(NO_3)_3$	53-60	43 d	Internal organs	6	270	164.2	608	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	43 d	Muscle	6	300	0.2	0.8	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	43 d	Skeleton	6	300	1.1	3.7	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	43 d	Gill	6	300	4.1	13.5	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$La(NO_3)_3$	53-60	43 d	Internal organs	6	300	180.6	602	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$SmCl_3$	53-60	43 d	Muscle	6	250	0.3	1.1	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$SmCl_3$	53-60	43 d	Skeleton	6	250	1.4	5.5	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$SmCl_3$	53-60	43 d	Gill	6	250	4	16	(Sun et al., 1996)
Fish	*Cyprinus carpio*	La	$SmCl_3$	53-60	43 d	Internal organs	6	250	176.3	705	(Sun et al., 1996)
Fish	*Danio rerio*	Ce	CeO_2 nanoparticles	97.7	7 d	Liver	7.2	500	1350[c]	2700[e]	(Johnston et al., 2010)

*All references are primary data
[a]Concentrations are nominal and total of lanthanide in the exposure medium
[b]Tissue concentration is expressed in wet weight, except otherwise described
[c]BCF is calculated from total or nominal REE concentrations in the water and expressed in wet weight, except otherwise described
[d]BCF is dynamic i.e., accumulation not in equilibrium yet, is calculated as described in Weltje et al (2002) and expressed based on fresh weight of plant.
[e]Values are calculated from dry weight of tissue.

Table 8. Bioaccumulation of lanthanides by saltwater organisms in field sites

Organism	Species	Base element	Polluted / Unpolluted	Tissue	Tissue conc. (μg REE g^{-1} dw)	Reference*
Cephalopod	*Nautilus macromphalus*	La	Unpolluted	Digestive Gland	0.3	(Pernice et al., 2009)
Cephalopod	*Nautilus macromphalus*	La	Unpolluted	Pericardial appendages	0.1	(Pernice et al., 2009)
Cephalopod	*Nautilus macromphalus*	Ce	Unpolluted	Digestive Gland	0.3	(Pernice et al., 2009)
Cephalopod	*Nautilus macromphalus*	Ce	Unpolluted	Pericardial appendages	0.2	(Pernice et al., 2009)
Cephalopod	*Nautilus macromphalus*	Nd	Unpolluted	Digestive Gland	0.2	(Pernice et al., 2009)
Cephalopod	*Nautilus macromphalus*	Nd	Unpolluted	Pericardial appendages	0.2	(Pernice et al., 2009)
Cephalopod	*Nautilus pompilius*	La	Unpolluted	Digestive Gland	1	(Pernice et al., 2009)
Cephalopod	*Nautilus pompilius*	La	Unpolluted	Pericardial appendages	0.2	(Pernice et al., 2009)
Cephalopod	*Nautilus pompilius*	Ce	Unpolluted	Digestive Gland	1.6	(Pernice et al., 2009)
Cephalopod	*Nautilus pompilius*	Ce	Unpolluted	Pericardial appendages	0.4	(Pernice et al., 2009)
Cephalopod	*Nautilus pompilius*	Nd	Unpolluted	Digestive Gland	0.9	(Pernice et al., 2009)
Cephalopod	*Nautilus pompilius*	Nd	Unpolluted	Pericardial appendages	0.3	(Pernice et al., 2009)
Mussel	*Mytilus edulis*	La	Unpolluted	Kidney	0.4	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	La	Unpolluted	Digestive Gland	0.3	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	La	Unpolluted	Gills	0.2	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	La	Unpolluted	Mantle	0.2	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	La	Unpolluted	Foot	0.1	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	Ce	Unpolluted	Kidney	0.5	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	Ce	Unpolluted	Digestive Gland	0.5	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	Ce	Unpolluted	Gills	0.2	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	Ce	Unpolluted	Mantle	0.2	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	Ce	Unpolluted	Foot	0.1	(Lobel et al., 1991)
Mussel	*Mytilus edulis*	La	Unpolluted	Soft tissue	3.7	(Riget et al., 1996)
Mussel	*Mytilus edulis*	Ce	Unpolluted	Soft tissue	4.7	(Riget et al., 1996)
Mussel	*Mytilus edulis*	Eu	Unpolluted	Soft tissue	0.02	(Riget et al., 1996)
Scallop	*Chlamys varia*	Ce	Polluted	Digestive Gland	10.6	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Polluted	Kidney	1.9	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Polluted	Gills	5.4	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Polluted	Gonad	5.7	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Polluted	Muscle	0.3	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Polluted	Non-organ tissue	4	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Unpolluted	Digestive Gland	2.2	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Unpolluted	Kidney	0.2	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Unpolluted	Gills	0.1	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Unpolluted	Gonad	1.5	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Unpolluted	Muscle	0.04	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Ce	Unpolluted	Non-organ tissue	0.6	(Bustamante and Miramand, 2005)

Table 8. Bioaccumulation of lanthanides by saltwater organisms in field sites (con't)

Organism	Species	Base element	Polluted / Unpolluted	Tissue	Tissue conc. (μg REE g^{-1} dw)	Reference*
Scallop	*Chlamys varia*	La	Polluted	Digestive Gland	7.9	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Polluted	Kidney	1.8	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Polluted	Gills	4	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Polluted	Gonad	5.1	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Polluted	Muscle	0.3	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Polluted	Non-organ tissue	2.8	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Unpolluted	Digestive Gland	0.2	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Unpolluted	Kidney	0.2	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Unpolluted	Gills	0.4	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Unpolluted	Gonad	0.7	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Unpolluted	Muscle	0.03	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	La	Unpolluted	Non-organ tissue	0.2	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Polluted	Digestive Gland	5.4	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Polluted	Kidney	0.7	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Polluted	Gills	2	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Polluted	Gonad	3.5	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Polluted	Muscle	0.1	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Polluted	Non-organ tissue	1.8	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Unpolluted	Digestive Gland	0.9	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Unpolluted	Kidney	0.1	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Unpolluted	Gills	0.3	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Unpolluted	Gonad	1.1	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Unpolluted	Muscle	0.01	(Bustamante and Miramand, 2005)
Scallop	*Chlamys varia*	Nd	Unpolluted	Non-organ tissue	0.3	(Bustamante and Miramand, 2005)

*All references are primary data

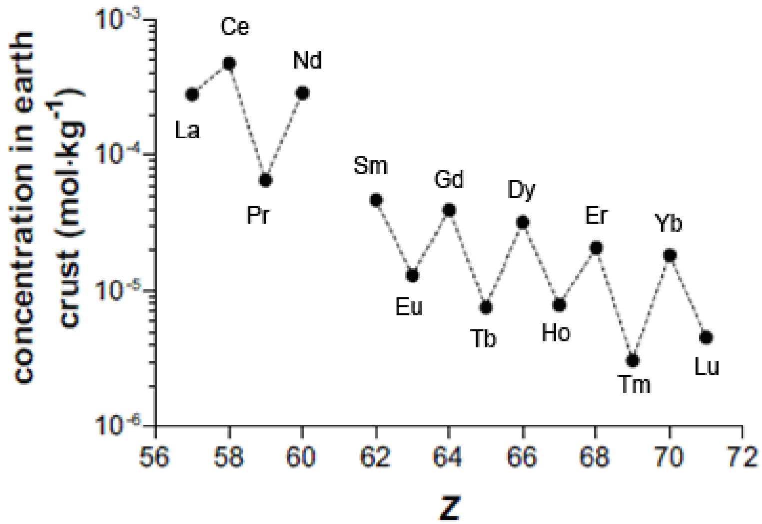

Fig. 1. Concentration (mol kg^{-1}) of lanthanides in the earth crust versus atomic number (Z). Data is obtained from Lide (Lide, 1994) and graph is modified from Weltje (Weltje, 2002). Pm has no stable or long-lived isotopes, hence no natural concentration is shown on the graph.

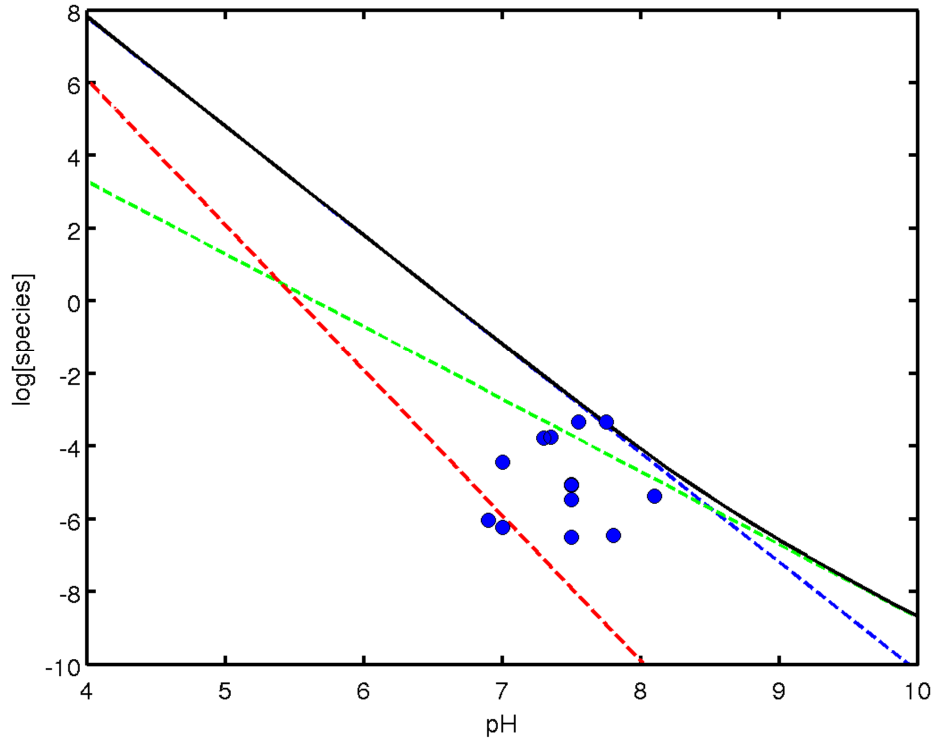

Fig. 2. Solubility of $La(OH)_{3(s)}$. The black line corresponds to equilibrium between $La(OH)_{3(s)}$ and aqueous solution. Above this line Lanthanum hydroxide is supersaturated. The blue lines corresponds to $[La^{3+}]$ in equilibrium with solid, green line is $LaOH^{2+}$ and the red line is $La_2OH_2^{2+}$ in eqiulibrium with the solid phase. The blue dots are La toxicity endpoints and pH values from Table 4 of this report.

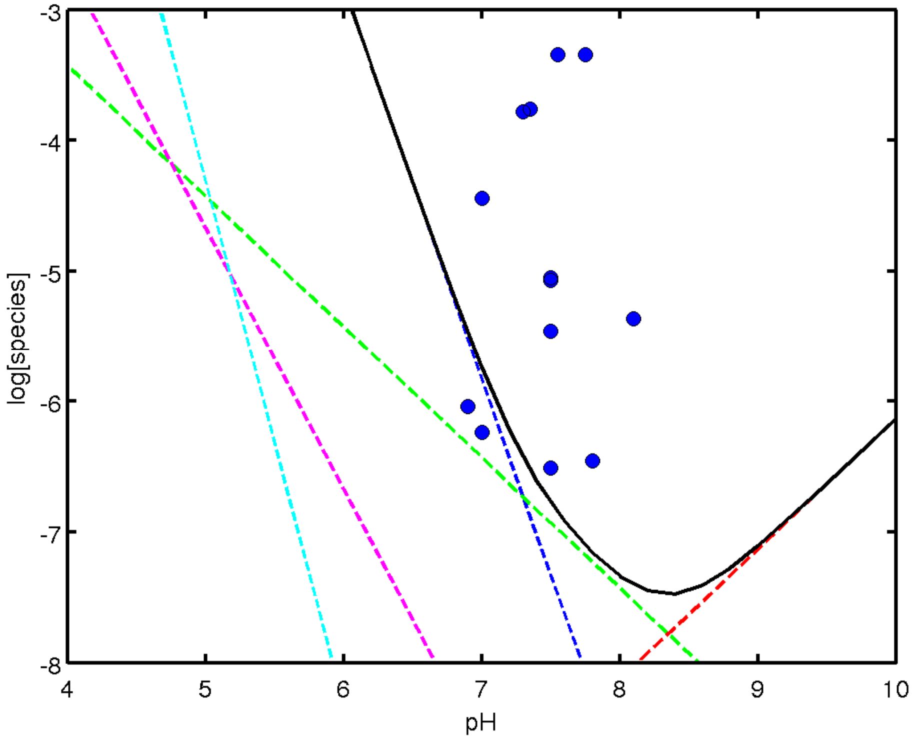

Fig. 3. Solubility of $La_2(CO3)_{3(s)}$. The black line corresponds to equilibrium between $La_2(CO_3)_{3(s)}$ and aqueous solution. Above this line Lanthanum carbonate is supersaturated. The blue lines corresponds to $[La^{3+}]$ in equilibrium with solid, green line is $[LaCO_3^{+}]$ and the red line is $[La(CO_3)_2^{-}]$ in eqiulibrium with the solid phase. The magenta line corresponds to $[LaHCO_3^{2+}]$ and the cyan line corresponds to $[La_2CO_3^{4+}]$.The blue dots are La toxicity endpoints and pH values from Table 4 of this report.

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Appendix A.

Derivation of solubility curves for La

The potential precipitation of two solid phases, hydroxide and carbonate, are investigated. The calculations presented below use thermodynamic constants from NIST (Martell and Smith, 2004). In all cases the lowest available ionic strength value was selected and temperature of 25 °C. The calculations assume the solid phase is in equilibrium with all dissolved species. Lanthanum is selected as the test lanthanide for this analysis.

0.1 Lanthanum Hydroxide Precipitation

For the dissolution reaction:

$$\mathrm{La(OH)}_{3(s)} = \mathrm{La}^{3+} + 3\mathrm{OH}^-$$

with known solubility product (K_{sp}) and dissociation constant for water (K_w), the concentration of La^{3+} versus pH can be calculated as follows:

$$
\begin{aligned}
K_{sp} &= [\mathrm{La}^{3+}][\mathrm{OH}^-]^3 \\
K_{sp} &= \frac{[\mathrm{La}^{3+}]Kw^3}{[\mathrm{H}]^3} \\
\log K_{sp} &= \log[\mathrm{La}^{3+}] + 3\log Kw - 3\log[\mathrm{H}^+] \\
\log[\mathrm{La}^{3+}] &= \log K_{sp} - 3\log Kw + 3\log[\mathrm{H}^+] \\
\log[\mathrm{La}^{3+}] &= \log K_{sp} - 3\log Kw - 3pH
\end{aligned}
$$

This is a linear equation determining $\log[\mathrm{La}^{3+}]$ as a function of pH with slope -3 and intercept $\log K_{sp} - 3\log Kw$.

Similarly the concentration of $\log \mathrm{LaOH}^{2+}$ can be calculated from the association constant (β_1)

$$
\begin{aligned}
\beta_1 &= \frac{[\mathrm{LaOH}^{2+}]}{[\mathrm{La}^{3+}][\mathrm{OH}^-]} == \frac{[\mathrm{LaOH}^{2+}][\mathrm{H}^+]}{[\mathrm{La}^{3+}]K_w} \\
\log[\mathrm{LaOH}^{2+}] &= \log\beta_1 + \log Kw + \log[\mathrm{La}^{3+}] + pH
\end{aligned}
$$

after substitution of log $[La^{3+}]$ expression

$$\log[LaOH^{2+}] = \log\beta_1 + \log Kw + (\log K_{sp} - 3\log Kw - 3pH) + pH$$

$$\log[LaOH^{2+}] = \log\beta_1 + \log K_{sp} - 2\log Kw - 2pH$$

The final NIST reported lanthanum hydrolysis product is $La_2OH_2^{4+}$. The symbol for the hydroxide formation constant can be given as β_{22} and the concentration can be derived as a function of pH:

$$\log[LaOH^{2+}] = \log\beta_{22} + 2\log K_{sp} - 4\log Kw - 4pH$$

Figure (XX) in the body of the report was derived using these equations and the following equilibrium constant values:

symbol	reaction	logK	ionic strength (M)
K_{sp}	$La(OH)_{3(s)}$=La^{3+}+$3OH^-$	-22.2	0
β_1	$La^{3+} + OH^- = LaOH^{2+}$	5.5	2.0
β_{22}	$2La^{3+} + 2OH^- = La_2OH_2^{4+}$	10.5	2.0

Table 1: Hydrolysis constants for Lanthanides. Values from NIST (Martell and Smith, 2004). All values 25 °C.

0.2 Lanthanum Carbonate Precipitation

For the dissolution reaction:

$$La_2(CO_3)_{3(s)} = 2La^{3+} + 3CO_3^{2-}$$

with known solubility product (K_{sp}) and dissociation constant for water (K_w), the concentration of La^{3+} versus pH can be calculated as follows:

$$\log[La^{3+}] = \frac{\log K_{sp} - 3\log[CO_3^{2-}]}{2}$$

Carbonate concentration can be calculated as a function of pH by assuming a fixed partial pressure of $CO_{2(g)}$ and associated carbonate equilibria:

$$\log[CO_3^{2-}] = \log K_{a1} + \log K_{a2} + \log K_H + \log P_{CO_2} + 2pH$$

Using these La^{3+} and CO_3^{2-} relationshiops, additional carbonate complexes can be calculated as ...

$$\log[LaCO_3^+] = \log\beta_1 + \log[La^{3+}] + \log[CO_3^{2-}]$$

$$\log[La(CO_3)_2^-] = \log\beta_2 + \log[La^{3+}] + 2\log[CO_3^{2-}]$$

$$\log[La_2(CO_3)^{4+}] = \log\beta_{21} + 2 * \log[La^{3+}] + \log[CO_3^{2-}]$$

Bicarbonate complex can be calculated using the following derived relationships ...

$$\log[LaHCO_3^{2+}] = \log\beta_{H1} + \log[La^{3+}] + \log[HCO_3^-]$$

$$\log[HCO_3^-] = \log K_{a1} + \log K_H + \log P_{CO_2} + pH$$

symbol	reaction	logK	ionic strength (M)
K_{sp}	$La_3CO_{32(s)}$=$3La^{3+}$+$2CO_3^{2-}$	-34.4	0.0
β_1	La^{3+} + CO_3^{2-} = $LaCO_3^+$	6.98	0.0
β_2	La^{3+} + $2CO_3^{2-}$ = $La(CO_3)^-$	11.86	0.0
β_{H1}	La^{3+} + HCO_3^- = $LaHCO_3^{2+}$	1.41	3.0
β_{21}	$2La^{3+}$ + CO_3^{2-}= $La_2CO_3^{4+}$	6.92	3.0
K_H	CO_2+H_2O=H_2CO_3	-1.47	0.0
K_{a1}	H_2CO_3=H^++HCO_3^-	-6.35	0.0
K_{a2}	HCO_3^-=H^++CO_3^{2-}	-10.33	0.0

Table 2: Carbonate equilibria and Lanthanide. Values from NIST (Martell and Smith, 2004). All values 25 °C.

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