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Howe Sound
Environmental Effects Monitoring (EEM)
Cycle Five Interpretive Report

Final

March 2010

Prepared for:

Howe Sound Pulp and Paper Limited Partnership (HSLP)
Port Mellon, British Columbia

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HOWE SOUND

ENVIRONMENTAL EFFECTS MONITORING (EEM) CYCLE FIVE INTERPRETIVE REPORT

FINAL

Prepared for:

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EXECUTIVE SUMMARY

The Environmental Effects Monitoring (EEM) Cycle Five program for Howe Sound Pulp and Paper Limited Partnership (HSLP) ran between April 2007 and April 2010, and included process effluent sublethal toxicological testing, fish tissue survey, and fish population survey components.

Ongoing process changes since the late 1980's have resulted in significant removal of TSS, BOD, AOX, and dioxins/furans in effluent being discharged to Howe Sound. Sublethal toxicity testing during Cycle Five demonstrated no effects of mill effluent on survival or growth of topsmelt (*Atherinops affinis*) larvae, effects on echinoderm fertilization at a mean effluent concentration of 32.8% (IC25), and effects on *Champia parvula* reproduction at a mean effluent concentration of 2.7% (IC25). Based on a 1% effluent concentration zone of 2,000 m from the diffuser, maximum potential zones of sublethal effect from the effluent discharge point were <20 m for fish growth and survival, 61 m for invertebrate fertilization, and 743 m for algal reproduction.

Dioxin and furan concentrations in sediments, crab, and fish near the HSLP mill have dropped considerably following pollution abatement changes in the 1980's and 1990's, including the phase out of elemental chlorine bleaching and introduction of secondary effluent treatment. Since the early 1990's, despite the complete elimination of elemental chlorine in 1997, concentrations of dioxins and furans in sediments have persisted near the diffuser, have not exhibited any recent patterns over time, and appear to be fluctuating at low levels. In 2009 this trend continued, and concentrations of 2,3,7,8-T_4CDD, 2,3,7,8-T_4CDF, and Total Toxic Equivalency (TEQ) were within the lower end of the historical range. In Dungeness crab, total TEQ concentrations at three of the eight sampling stations were above Health Canada's consumption threshold (24.4 pg/g hepatopancreas), while levels at the remaining five stations were below the threshold. The total TEQ concentration in dogfish liver was over 34 times greater than the threshold (30.0 pg/g liver), and the concentration in liver from a single English sole was below the consumption threshold.

An extended 28-day topsmelt sublethal toxicity test was performed for the HSLP EEM Cycle Five fish survey. The effluent dilution series proposed in the design document for Cycle Five was a repeat of the Cycle Three series of 1%, 2.5%, 6.4%, 16%, 40%, and 100% effluent. Unfortunately, the dilution series used by the laboratory for the test was a 0.5-factor dilution series of 6.25%, 12.5%, 25%, 50%, and 100% effluent. Following discussions with Environment Canada, the test was not repeated using the correct dilution series given that the original test failed due to poor survivorship of embryos/larvae in the control treatment and there was no evidence of a dose response among treatments using the 0.5-factor dilution series.

High mortality rates of the control (36%) in exceedance of the maximum allowable rate (35%) were reached by Day 13 of the 28 day test. High mortality rates and inconsistent dose response were also associated with the majority of effluent test concentrations. Due to mortality rates for both the control and effluent test concentrations, the test was terminated after Day 13. The testing laboratory was unable to identify the specific cause of high mortality rates; however, sensitivity of organisms to handling (particularly during

hatch) was identified as a potential reason for the test's failure. The lab was also not able to identify any additional precautions or procedures that could have further minimized mortality rates. In general, marine fish egg tests are sensitive, often perform poorly and unpredictably, and are not routinely used by ecotoxicology labs.

The HSLP Local Monitoring Committee (LMC), on advice from EEM Science Committee members, explored potential alternatives for completing the Cycle Five fish survey following failure of the extended topsmelt sublethal toxicity test. Alternative bioassays proposed by the ecotoxicology lab included a bivalve larval development test, an echinoderm larval development test, and a rainbow trout egg-alevin test. In addition, the EEM Science Committee proposed either repeating the extended topsmelt test or performing an expanded benthic survey as a fish study alternative.

After much deliberation, LMC and EEM Science Committee members agreed that none of the proposed alternatives were suitable or feasible for implementation in Cycle Five. The primary concern with the substitute laboratory bioassays was that they would not adequately answer the EEM fish survey question regarding potential local fish population effects associated with mill effluent. Bivalve and echinoderm tests would have provided similar results to the routine sublethal toxicity program, while the freshwater rainbow trout species test would not have been particularly relevant to the marine environment. The ecotoxicology lab repeating not repeating the extended topsmelt test, given the likelihood that a similar mortality rate would occur.

The extended benthic invertebrate survey, although the most reasonable alternative, was not selected for implementation given the lack of remaining time available in Cycle Five and because a similar study was just completed in Cycle Four. An expanded benthic invertebrate survey will be explored as a possible alternative approach to the fish survey in Cycle Six.

1.0 INTRODUCTION

Under the federal *Pulp and Paper Effluent Regulations* (originally released in 1992, revised in May 2004 (Government of Canada 2004), and updated in 2008) pulpmills are required to monitor the chemistry and toxicity of mill effluent and its potential effects on the receiving environment. Effluent chemistry (limited to total suspended solids and biological oxygen demand) and lethal toxicity are measured to evaluate effluent quality and its potential effects on aquatic biota. However, because there are many factors that can alter the chemistry and toxicity of effluent in the receiving environment, Environmental Effects Monitoring (EEM) studies are also conducted to directly assess the effects of mill effluent on fish, fish habitat, and use of fisheries resources in the vicinity of the effluent discharge (Environment Canada 2005). EEM studies can include:

- A fish population survey to assess fish health;
- A fish tissue survey to assess concentrations of dioxins and furans (only required for mills where dioxins and furans are present in mill effluent);
- A benthic invertebrate community survey to assess the condition of fish habitat;
- Supporting water quality data to help interpret findings from fish and benthic invertebrate surveys; and
- Sublethal toxicity testing to assess effects of effluent on growth and reproduction of representative aquatic organisms.

EEM programs typically are conducted in three year cycles, which begin with the development of a study design, followed by study implementation, data analysis, and reporting. The following cycles have been completed for the HSLP Mill since the onset of the monitoring program:

- Cycle One: 1993 to 1996;
- Cycle Two: 1997 to 2000;
- Cycle Three: 2000 to 2004; and
- Cycle Four: 2004 to 2007.

The current program, Cycle Five, ran between April 2007 and April 2010. All components of the EEM program were conducted in accordance with the *Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring*. The 2005 technical guidance document remains applicable to Cycle 5 (Environment Canada 2005), although updated guidance based on the 2008 PPER amendments is in progress.

This report presents results from the EEM Cycle Five program for HSLP. The program, previously described in the study design (Hatfield Consultants 2009a), included a fish survey (using an extended 28 day topsmelt sublethal toxicity test), fish tissue study (crabs, finfish, and sediments), and sublethal toxicity testing of mill effluent. Information on changes to mill processes, effluent treatment, and/or the receiving environment that occurred during Cycle Five also is presented. Sections in this report include:

- Section 2 – Mill, Study Area, and Cycle Five Design Update;
- Section 3 – Sublethal Toxicity Testing of Mill Effluent;
- Section 4 – Fish Population Survey;
- Section 5 – Fish Tissue Survey;
- Section 6 – Conclusions;
- Section 7 – Closure;
- Section 8 – References;
- Section 9 – Glossary; and
- Appendices.

2.0 MILL, STUDY AREA, AND CYCLE FIVE DESIGN UPDATE

2.1 MILL OPERATIONS

2.1.1 Process Description and Update

HSLP operates a pulp and paper mill on Thornbrough Channel near Port Mellon in Howe Sound, British Columbia, Canada (Figure 2.1). The mill is located on the mainland directly across from the western side of Gambier Island. The mill has been in operation since 1909, and currently produces bleached Kraft pulp and newsprint.

The mill's wood furnish varies according to the pulp grade. In general, blends of hemlock, fir, cedar, and SPF (spruce, pine, fir) are used. Various sawmills from the surrounding area and the Lower Mainland supply woodchips.

Total production in 2009 was 473,018 ADMt, average production was 1,515 ADMt/d, and average effluent flow was 70,700 m^3/day (Table 2.1; Figure 2.2).

Detailed descriptions of mill processes, including bleaching, are documented in the Cycle One pre-design report (Hatfield 1994). Some of the key process updates that have occurred in recent years include:

- implementation of the current outfall configuration in 1981 whereby process effluent is discharged via six ports along a deep-sea diffuser ranging from 30 to 115 m depth;
- introduction of high levels of chlorine dioxide substitution in 1989 (50% and greater substitution);
- installation of a UNOX secondary effluent treatment system (activated sludge breakdown using microorganisms) in 1990; and
- complete elimination of elemental chlorine bleaching at the mill in 1997.

Operational updates that occurred at the mill during Cycle Five included:

- temporary curtailment of paper mill production for a total of 99 days in 2009, including: April 13 to 20, June 6 to 15, July 22 to 27, August 17 to September 16, October 13 to 28, November 18 to December 1, December 23 to January 5, March 11 to Sept 30, 2009; and
- temporary curtailment of kraft mill production for a total of 32 days in 2008, including: November 17 to December 1 and December 21 to January 6.

Table 2.1 Annual mean values for process effluent quality variables, Howe Sound Pulp and Paper Limited Partnership, 2007 to 2009.

Variable	2007	2008	2009
Production			
Total Production (ADt/day)	1,588	1,568	1,515
Total Production (ADt/yr)	550,708	532,669	473,018
Kraft Pulp (ADt/yr)	362,151	321,220	323,787
Newsprint (ADt/yr)	188,557	211,449	149,231
Treated Effluent Quality			
Effluent Flow (m^3/d)	73,000	72,300	70,700
pH	6.4	6.4	6.4
TSS (kg/day)	1,916	2,273	1,784
$BOD_{5\ day}$ (kg/day)	896	981	634
AOX (kg/day)	261	288	231
AOX (kg/ADt)	0.21	0.29	0.24
Toxicity (LC50)[1]:			
Daphnia Magna (48h LC50)	100%	96%	100%
Rainbow Trout (96h LC50)	93%	100%	100%
2,3,7,8-T_4CDD (ppq)	ND	ND	ND
2,3,7,8-T_4CDF (ppq)	ND	ND	ND

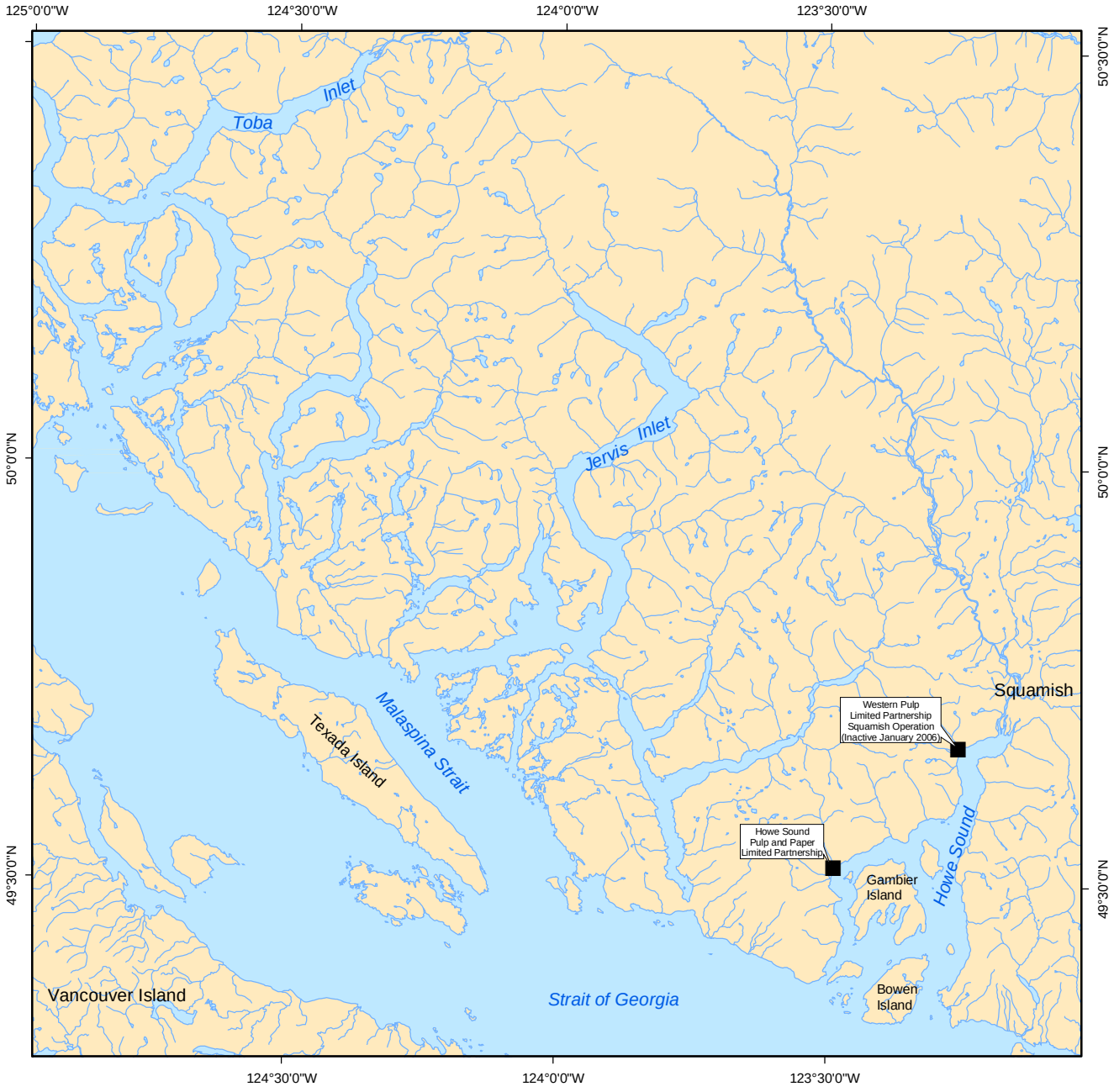
ND = Not Detected.

ADt = air dried metric tonnes.

ppq = parts per quadrillion (pg/L).

[1] Percentage of tests conducted where LC50 (effluent concentration that kills 50% of organisms) was >100%.

Figure 2.1 Location of Howe Sound Pulp and Paper Limited Partnership on Thornbrough Channel, Howe Sound, British Columbia.



LEGEND

■ Mill

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0 5 10 20 km

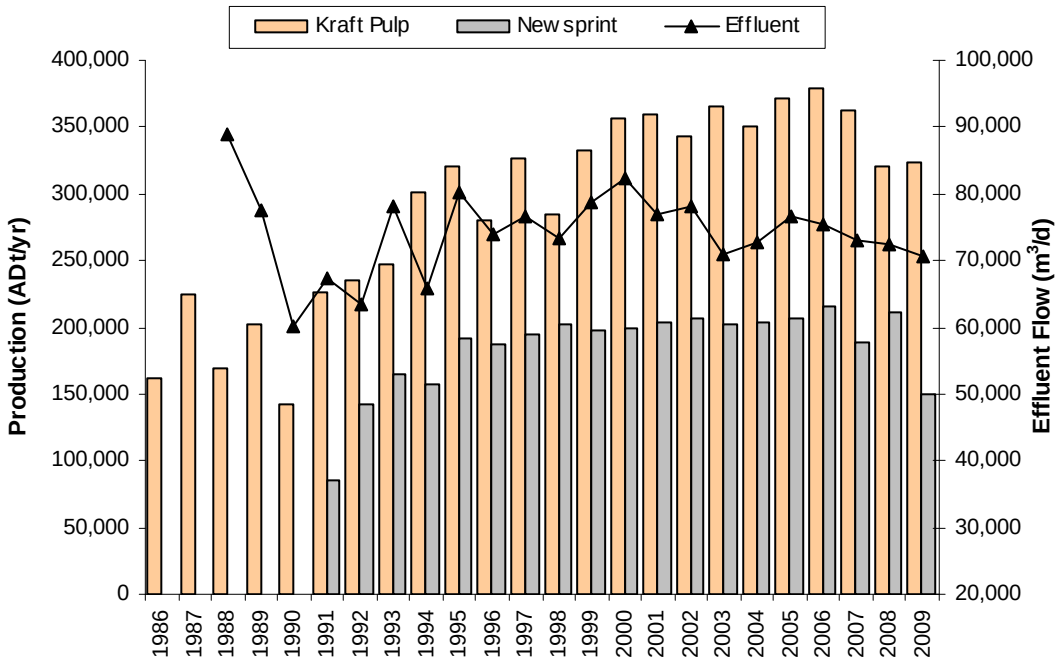
Scale 1:850,000

Projection: UTM Zone 10 NAD83

N

K:\Data\Project\HS1325_MXD\B_Interpret\HS1325_B01_Location_20100111.mxd

Figure 2.2 Annual pulp production and effluent flow from 1986 to 2009, Howe Sound Pulp and Paper Limited Partnership.



2.1.2 Effluent Quality

HSLP discharges treated final effluent from Kraft and newsprint processes via six ports located along a deep-sea diffuser ranging from 30 to 115 m depth, extending 277 m into Thornbrough Channel in Howe Sound.

Effluent quality variables are routinely measured following provincial and federal requirements; annual results are presented in Table 2.1 for Cycle Five (i.e., 2007 to 2009). Mean annual pulp production levels at the mill steadily increased between 1991 and 1997, and then began to decline in 2007 due to significant mill downtime; effluent discharge rates have generally declined since 2000 (Figure 2.2).

Total suspended solids (TSS), biological oxygen demand (BOD), and concentrations of adsorbable organic halides (AOX) in effluent declined initially following introduction of chlorine dioxide in 1989, and again after the installation of secondary effluent treatment in 1990 (Figure 2.3). AOX declined again after the complete elimination of elemental chlorine bleaching in 1997. In recent years, levels of all three variables have remained relatively consistent. In 2009, TSS was 1,784 kg/day, BOD was 634 kg/day, and AOX was 0.24 kg/ADt (Table 2.1).

Tetra-chlorinated dioxins (i.e., 2,3,7,8-T4CDD) and furans (i.e., 2,3,7,8-T4CDF) were not detected in HSLP effluent at any time during Cycle Five (Table 2.1).

In order to remain in compliance with the federal *Pulp and Paper Effluent Regulations* (PPER), mills are required to sustain no acute toxicity of effluent to rainbow trout (i.e., all LC50s – effluent concentrations that kill 50% of trout – must be greater than 100% v/v effluent). In 2007, acute toxicity (96-hour LC50) endpoints for rainbow trout passed (LC50 > 100%) 93% of the time; one test failed due to high residual ammonia. Rainbow trout tests passed 100% of the time in 2008 and 2009 (Table 2.1). *Daphnia magna* 48-hr acute toxicity tests passed 100% of the time in 2007, 96% of the time in 2008, and 100% of the time in 2009.

2.1.3 Spills to the Receiving Environment

One spill to the receiving environment was reported by HSLP between 2007 and 2009 (Cycle Five): a small spill of untreated effluent of an unknown volume was discharged through Storm Sewer C during a power failure on August 6, 2008. The general sewer pumps shut down prior to the spill from what appeared to be a communications failure. When it was apparent that the pumps were not starting immediately, the mill's shutdown procedure began and berms were constructed to contain the effluent. Operations continued to troubleshoot the problem and were able to restart the pumps 30 minutes later. Through the course of these events, effluent made its way to Storm Sewer C. Additional berming was constructed and samples were collected. Fifteen minutes later, the situation was contained. It was difficult to estimate the volume discharged, however given the time that pumps were down and the length of time it would take for the sewers to flood, the amount would have been relatively small.

A preliminary investigation into the incident cited a failure of the UPS operating the control system of the pumps; an incident investigation was conducted, which confirmed this initial assessment.

The spill was reported to the Provincial Emergency Program and a report was sent to the Ministry of Environment detailing the spill, and no action was taken by any regulating body.

No other spills were reported in Cycle Five.

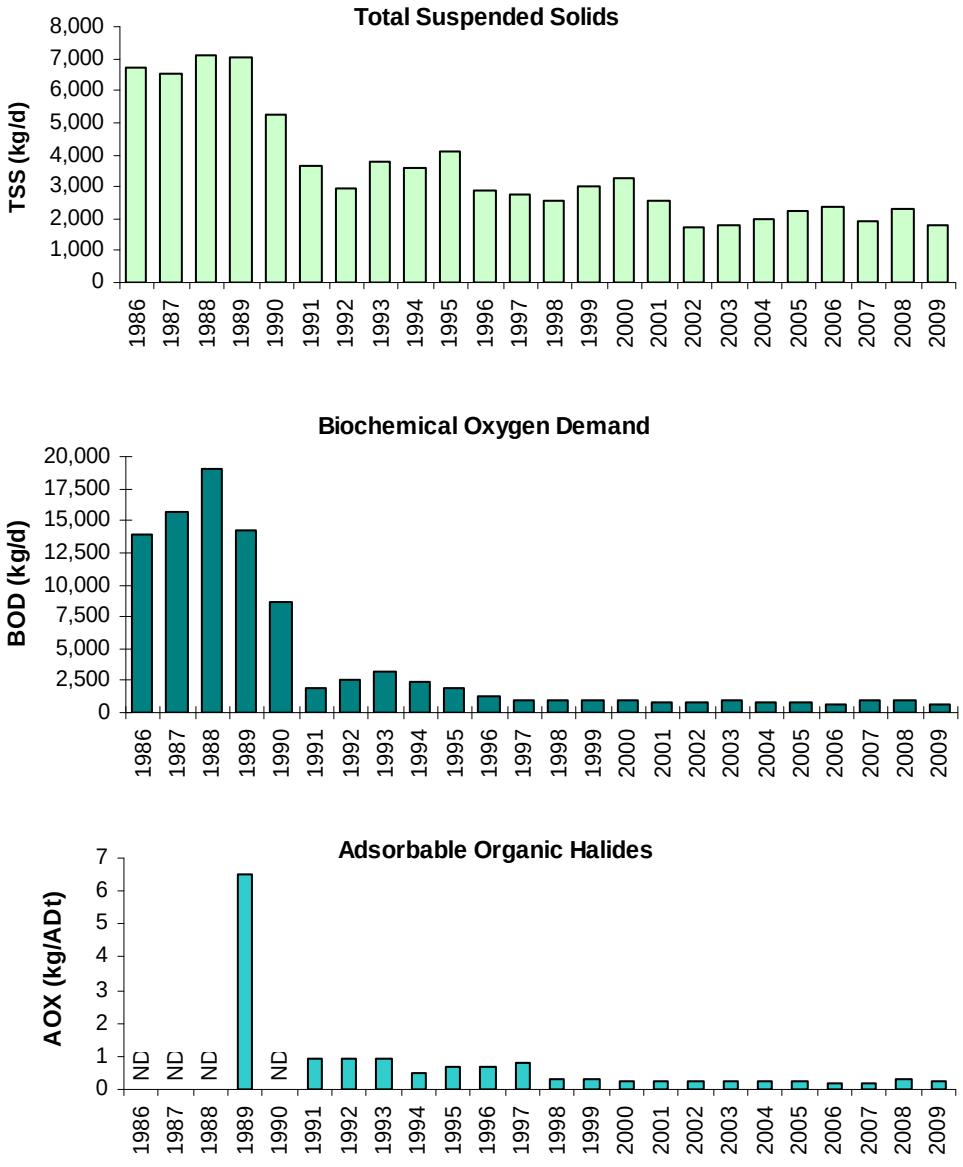
2.1.4 Study Area Updates

Construction of the Sea-to-Sky Highway continued through Cycle Five along the eastern banks of Howe Sound. It is unlikely that construction activities would result in any noticeable effect at any of the EEM sampling locations (i.e., dioxin/furan monitoring areas). During Cycle Five there were no other major anthropogenic influences or significant natural ecological variations in the HSLP EEM study area.

2.2 CYCLE FIVE STUDY DESIGN UPDATE

No major changes were made to the Cycle Five study design during field surveys.

Figure 2.3 Mean daily total suspended solids (TSS), biochemical oxygen demand (BOD), and adsorbable organic halides (AOX) in effluent, Howe Sound Pulp and Paper Limited Partnership, 1986 to 2009.



ND = No Data Available

3.0 SUBLETHAL TOXICITY TESTING OF MILL EFFLUENT

Summary of Sublethal Toxicity Testing for HSLP EEM Cycle Five:

- Topsmelt larvae growth (IC25) and survival (LC50) were not affected;
- Echinoderm fertilization was affected at a mean effluent concentration of 32.8% (IC25);
- *Champia* reproduction was affected at a mean effluent concentration of 2.7% (IC25); and
- Based on a 1% effluent concentration zone of 2,000 m from the HSLP diffuser, maximum potential zones of sublethal effect from the effluent discharge point were <20 m for fish growth and survival, 61 m for invertebrate fertilization, and 743 m for algal reproduction.

Federal and provincial government regulations require pulp and paper mills to undertake toxicity testing as part of their EEM programs to determine any lethality or inhibitory effects that their effluent may have on fish populations and fish habitat. Sublethal and acute lethality tests may indicate whether fisheries resources are being protected or impacted in areas adjacent to their effluent discharges. Current EEM regulations require the use of sublethal toxicity tests to help meet the following objectives:

- Contribute to the field program as part of a weight-of-evidence approach;
- Compare process effluent quality between mill types and measure changes in effluent quality as a result of effluent treatment and process changes; and
- Contribute to the understanding of relative contributions of the mill to multiple discharge situations (Environment Canada 2005).

For HSLP and other pulpmills which discharge to a marine receiving environment, Cycle Five sublethal toxicity testing included the following tests (Environment Canada 2005):

- Fish early life stage development growth and survival tests using topsmelt (*Atherinops affinis*). This test was excluded from the EEM testing requirements in August 2008 as stated in amendments to the *Pulp and Paper Effluent Regulations* (Government of Canada 2008);
- Invertebrate fertilization toxicity test using an echinoderm (either the sand dollar *Dendraster excentricus*, or purple sea urchin *Strongylocentrotus purpuratus*); and
- Plant reproduction toxicity test using a red marine alga (*Champia parvula*).

Sublethal toxicity testing was conducted by Cantest Ltd. (Burnaby, BC), except for the *Champia parvula* test, which was conducted by the Saskatchewan Research Council (Saskatoon, SK). A summary of reported endpoints is included in this report.

3.1 METHODS

3.1.1 General Methods and Definitions

Sublethal toxicity testing of process effluent must be conducted twice a year, during winter and summer terms, for a total of six test periods per three-year EEM cycle (Hatfield 2005). Testing for Cycle Five was initiated in winter 2007 and continued until summer 2009.

In Cycle Five, test seasons assigned were not necessarily representative of the date the test was conducted. The first test period of each year (the "winter" test period) was usually carried out in April or May. The second test period of each year (the "summer" test period) was usually carried out between September and November. The apparent discrepancy in the naming of the test seasons was due to delays that occurred in Cycle Three, resulting from retesting combined with the restrictions associated with test organism availability. Figures presented in this section provide both the test season name and actual test date to prevent any confusion. This naming discrepancy has not been corrected because it has no effect on the validity of toxicity results, and because correcting the naming would require that two sequential test periods be conducted too close to each other. The intent of having two test periods per year is to have them approximately six months apart (J. Boyd, Environment Canada, *pers. comm.,* 2006).

Mill personnel collected a grab sample of effluent on each test date according to the methodology described in the *Technical Guidance Document* (Environment Canada 2005). Sublethal toxicity tests involve exposure of organisms to a series of effluent dilutions. All sublethal toxicity tests were conducted with controls in order to assess the "background response" of test organisms and determine the acceptability of the test using predefined criteria. For test endpoints reported for EEM Cycle Five, controls must meet or exceed test protocol requirements. In addition, in-house cultures are tested with a reference toxicant to monitor the health and sensitivity of the culture.

The endpoints that are reported for these toxicity tests are LC50 or IC25 endpoints. The LC50 endpoint for survival is the effluent concentration that is lethal to 50% or more of the organisms tested. The IC25 endpoint is an estimate of the concentration of effluent that causes 25% impairment of a non-lethal endpoint, including growth (used for the fish tests), fertilization (invertebrate tests), and reproduction (algal tests). Confidence limits are given for each endpoint where possible, indicating overall variability in the endpoint.

A zone of effluent mixing was determined by a plume delineation study during the Cycle One pre-design study (Hatfield 1994). That study determined the maximum extent of effluent concentration of 1% (i.e., 100:1 dilution) or greater, potentially present in the receiving water environment. This 1% effluent zone originally was used to define near-field and far-field areas to aid in selecting sites to conduct required environmental sampling.

The 1% effluent zone represents conditions of minimum dilution, maximum extent, and long-term average conditions (i.e., long-term effect of effluent discharge) (Environment Canada 2005), and therefore represents worst-case effluent dilution conditions. HSLP's maximum extent of 1% effluent was defined as a radial distance of approximately 2 km from the diffusers (Hatfield 1994).

A maximum potential zone of sublethal effect was calculated for each test species from the geometric mean of the IC25, EC25, or LC50 endpoints and the extent of the 1% effluent concentration zone, as per Environment Canada (2005). This potential zone of sublethal effect describes the downstream area where the effluent concentrations exceed the geometric mean of the IC25, EC25, or LC50 endpoint, and is the maximum distance from the effluent discharge where a specified effect may be expressed for a test species. This maximum zone of potential sublethal effect was calculated as follows:

$$\text{Zone (m)} = \frac{\text{Extent of 1\% effluent zone (m)}}{\text{Geometric mean of IC25, EC25 or LC50 endpoints}}$$

As discussed in the Results and Discussion (Section 3.2.4), this model may not be realistic for the Howe Sound mill, given that effluent is discharged through multi-port diffusers that rapidly dilute effluent upon release.

3.1.2 Sublethal Toxicity Test Methods

General procedures for conducting the topsmelt tests were based on *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms*, First Edition, EPA/600/R-95/136, August 1995 (US EPA 1995). This seven-day static renewal test uses nine to 15-day-old topsmelt (*Atherinops affinis*) larvae to assess the toxicity of a sample by comparing the growth and survival of exposed organisms to that observed in control organisms. The endpoints are effluent concentrations that result in 50% survival for the seven days (LC50) and for 25% inhibition of growth measured as dry weight (IC25) relative to control weights.

General procedures for the echinoderm fertilization test are based on the methodology document *Biological Test Method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)*, Report EPS 1/RM/27, December 1992, November 1997 amendments (Environment Canada 1997b). The test assesses the fertilization success of an echinoderm using the sand dollar *Dendraster excentricus* or the sea urchin *Strongylocentrotus purpuratus*. Male and female gametes are exposed to the test material for 20 minutes; the percentage of eggs fertilized is compared between the controls and the sample concentrations to determine if any significant inhibition of fertilization is observed. The IC25 endpoint is the percent effluent concentration where fertilization is reduced by 25% from control fertilization rates.

Procedures for conducting the marine algae (*Champia parvula*) test are based on *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms*, Third Edition, EPA821/R/02-014,

October 2002 (US EPA 2002). The *Champia* test is a static, non-renewal, marine algal reproduction test where male and female plants are exposed to a test sample for a 48-hour period, followed by a six to eight-day recovery period. The inhibition of cystocarp reproduction by 25% by the end of the recovery period is the effluent concentration endpoint (reproduction IC25) used to assess toxicity.

3.2 RESULTS AND DISCUSSION

HSLP conducted six sublethal toxicity tests from winter 2007 through summer 2009 for Cycle Five. The summer 2008 and winter 2009 *Champia* tests were repeated due to poor culture health in the original tests. The winter 2007 echinoderm tests were also repeated because the seawater control failed to meet survival criteria. Appendix A1 provides a summary of Cycle Five sublethal toxicity test results, including dose-response plots for all tests conducted.

3.2.1 Topsmelt Growth and Survival Test

Topsmelt Growth and Survival tests were completed for the winter 2007 through winter 2008 test periods. Consistent with changes made to the *Pulp and Paper Effluent Regulations* (Government of Canada 2008), this test was no longer required after winter 2008. For HSLP effluent, the topsmelt LC50 (survival) and IC25 (growth) endpoints were >100% (i.e., the highest concentration of effluent tested resulted in no observed effect), indicating no toxicity during the Cycle Five testing period (Figure 3.1, Figure 3.2). These results are similar to what was observed in Cycle Four (Hatfield 2007).

Figure 3.1 Topsmelt early life stage survival LC50 endpoints and 95% confidence limits for HSLP, EEM Cycle Five.

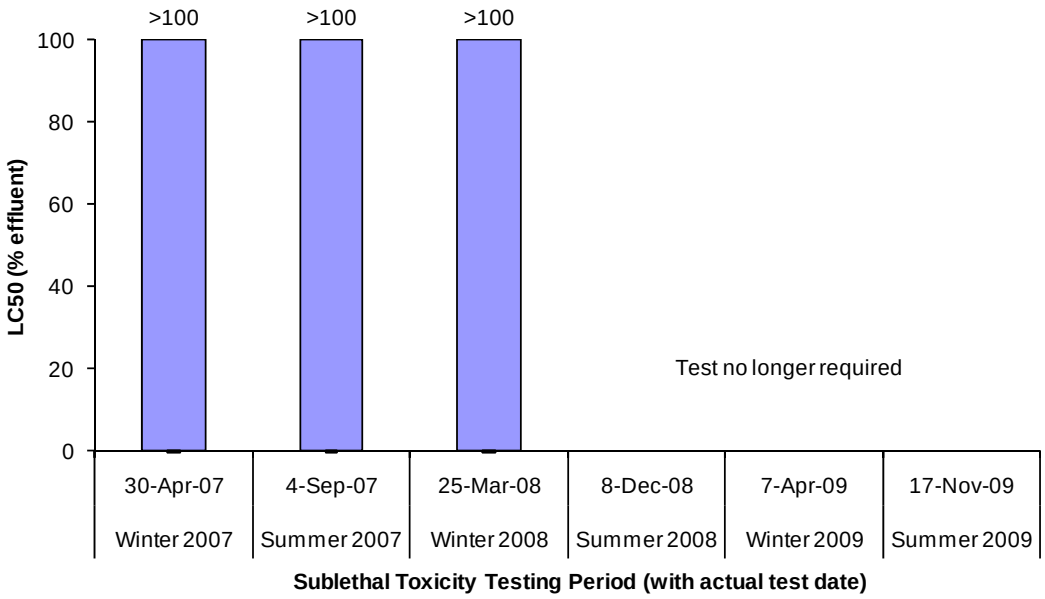
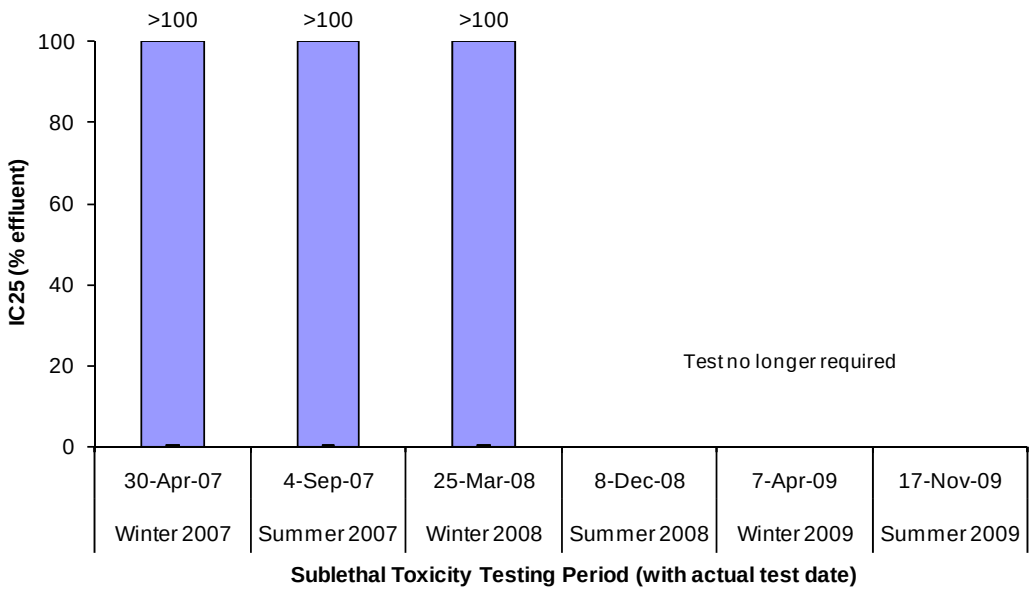


Figure 3.2 Topsmelt early life stage growth IC25 endpoints and 95% confidence limits for HSLP, EEM Cycle Five.



3.2.2 Echinoderm Fertilization Test

Echinoderm fertilization results for the summer 2006 test period (Cycle Four) are included in this report (Figure 3.3); these results were excluded from the Cycle Four report because brine controls failed the minimum survival criteria and results could not be obtained before finalizing the Cycle Four report. Echinoderm (sand dollars and sea urchins) fertilization IC25s ranged from 16.6 to 80.1% effluent in Cycle Five, with a geometric mean of 32.8%. Results were variable across test periods, but were similar to Cycle Four. Dose response curves were relatively consistent among testing periods (Appendix A1). The lowest IC25 (most toxicity) was reported in the winter 2009 period; however, a similar result was not observed in tests using other test species. Furthermore, no process upsets were reported and no noticeable change in effluent quality data were observed for the winter 2009 period. In general, the IC25 echinoderm fertilization endpoints showed a decreasing trend throughout most of Cycle Five, followed by an increase in the summer 2009 test period (Figure 3.3).

3.2.3 *Champia* Reproduction Test

Champia reproduction IC25 endpoints ranged from 1.31 to 5.77% with a geometric mean of 2.7%. Although lower individual results were observed in Cycle Four (i.e., one IC25 was lower [3.87%] than all results in the first half of Cycle Five), average results in Cycle Five indicated an increased effect of effluent on *Champia* reproduction. However, IC25 endpoints were variable during Cycle Five and no trends were apparent. The summer 2008 effluent sample resulted in the greatest effect while the winter 2007 test period effluent sample resulted in the smallest effect (Figure 3.4). Overall, IC25 endpoints and dose-response plots were relatively consistent over time (Appendix A1).

Figure 3.3 Echinoderm fertilization IC25 endpoints and 95% confidence limits for HSLP, EEM Cycle Five.

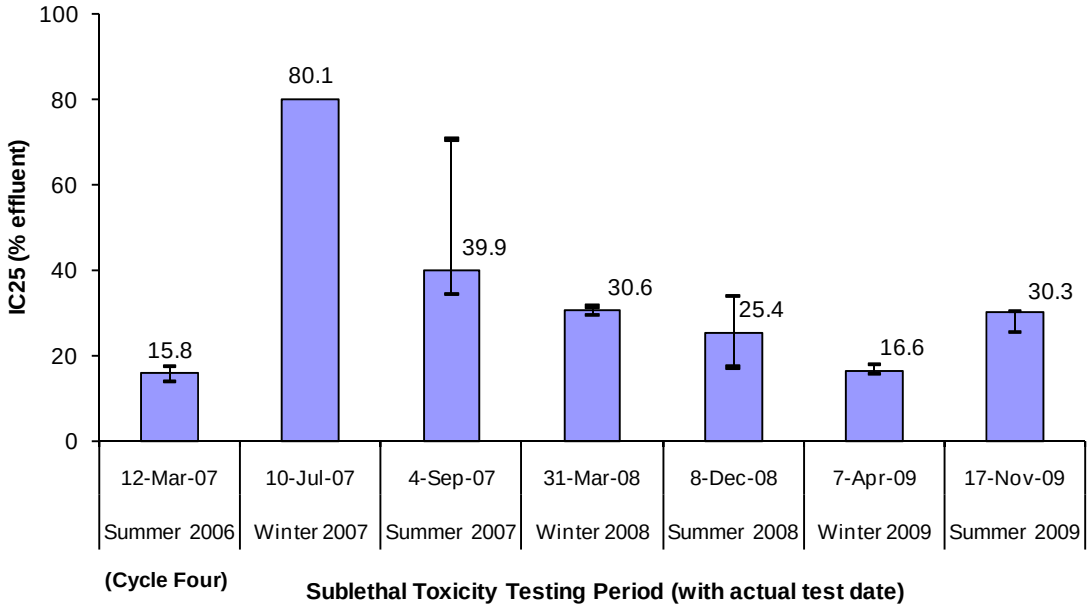
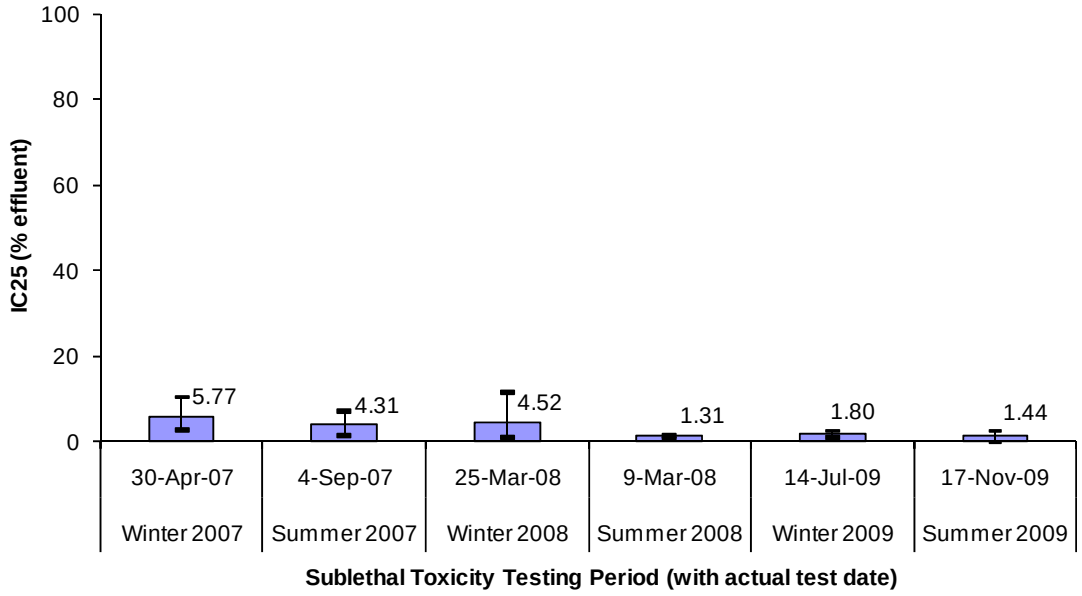


Figure 3.4 *Champia* reproduction IC25 endpoints and 95% confidence limits for HSLP, EEM Cycle Five.



3.2.4 Potential Zone of Sublethal Effect

The 1% zone of effluent concentration for HSLP extends approximately 2,000 m in radius from the effluent diffuser (Hatfield Consultants Ltd. 1994). Table 3.1 presents the geometric means of IC25 endpoints for each test species for all five cycles, and the resulting Potential Zone of Sublethal Effect calculated using the 2,000 m length for the 1% effluent zone. Calculations of geometric means and potential zones of sublethal effects can be found in Appendix A1.

The potential zone of sublethal effect for topsmelt cannot be calculated with any accuracy, as the IC25 concentrations were always greater than the highest concentration tested (i.e., >100% effluent). Consequently, the zone is shown as being less than the distance calculated, assuming that the IC25 was equal to the highest concentration tested.

The zone of sublethal effect decreased for echinoderms from 109 m in Cycle Four to 61 m in Cycle Five. The *Champia* zone of sublethal effect is the largest; this value has increased since Cycle One, signifying an increase in toxicity over time (Table 3.1).

Table 3.1 Potential Zone of Sublethal Effect, HSLP, EEM Cycles One through Five.

Sublethal Toxicity Test Species	IC25 or LC50 Geometric Mean (% v/v)					Potential Zone of Sublethal Effect [1] (m)				
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
***Atherinops affinis*[2,3]**										
Growth	67.7%	67.2%	77.9%	>100%	>100%	30m	30m	36m	<20m	<20m
Survival	67.7%	67.2%	77.9%	>100%	>100%	30m	30m	36m	<20m	<20m
Echinoderm Fertilization	11.1%	16.0%	25.0%	18.3%4	32.8%	180m	125m	80m	109m4	61m
***Champia parvula* Reproduction**	17.5%	19.1%	19.2%	10.2%	2.7%	114m	105m	104m	196m	743m

[1] Based on 1% effluent zone of 2,000m.

[2] *Menidia beryllina* was used as the fish test organism during Cycle One. One growth result of >31% was not used in calculation. Please refer to the Cycle One report for explanation.

[3] Cycle Five geomeans and Potential Zones of Sublethal Effect for Topsmelt growth and survival are based on three test periods. Testing for this species was no longer required after winter 2008.

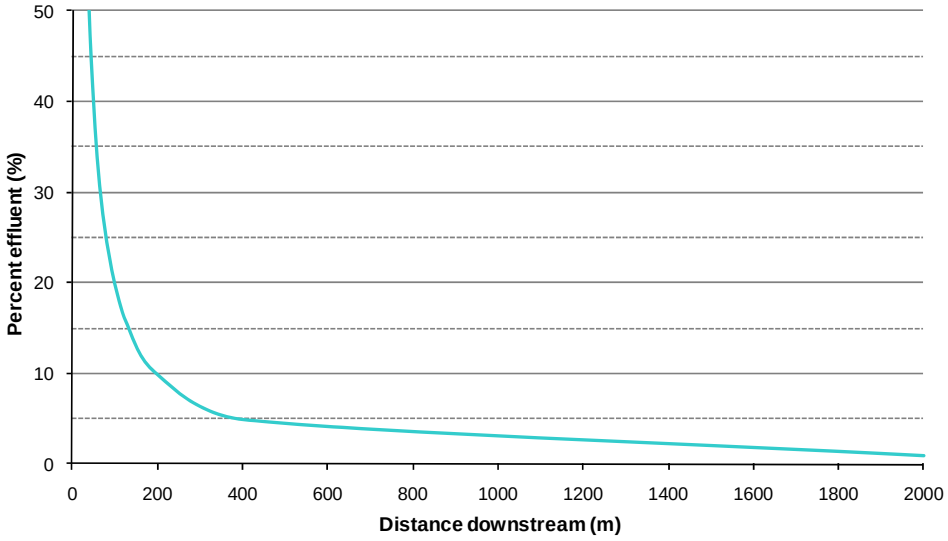
[4] Echinoderm Fertilization test results for the summer 2006 test period were excluded from this calculation in the Cycle Four Interpretive Report because retest results were unavailable at the time the report was finalized. Values for Cycle Four presented here have been updated to include these results.

In Cycle Five, the geometric mean of the IC25 endpoint for *Champia parvula* reproduction was equal to 2.7%. This is slightly above the 2.3% concentration value observed during an effluent dispersion study conducted in 1989 (Hodgins and Knoll 1990a). However, all other IC25 endpoint values calculated in Cycle 5 were higher than the observed value of 2.3%. No other major industrial or

municipal discharges occur within the study area for HSLP that could confound toxicity impacts in the near-field receiving environment. However, the presence of log booms likely has some ecological implications to the HSLP near-field receiving environment.

Figure 3.5 presents the rate of effluent dilution for HSLP implied by the Environment Canada (2005) zone-of-effect model. Using a conservative maximum extent of 1% effluent of 2000 m, this model assumes that effluent would be diluted to 50% of release concentration 40 m from the diffuser, to 20% of release at 100 m, and to 5% of release at 400 m from the diffuser. However, actual dilution of HSLP effluent occurs much more rapidly than this, mainly due to its discharge through a multi-port diffuser, which was designed to maximize initial dilution and dispersion of effluent in the marine receiving environment.

Figure 3.5 Dilution of HSLP effluent in the marine receiving environment assumed by the Environment Canada (2005) maximum-zone-of-effect model.



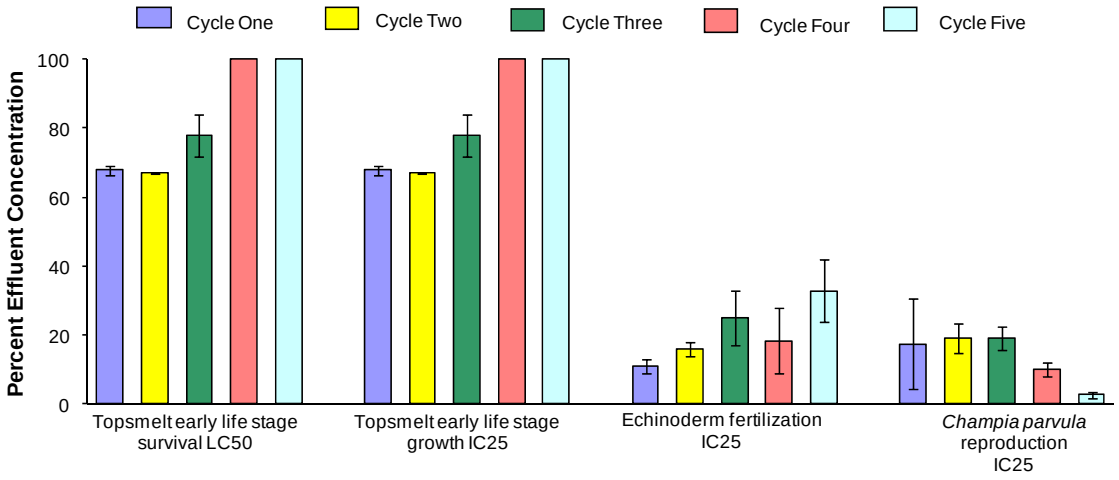
3.3 CONCLUSIONS

The toxicity testing results contribute to the overall weight-of-evidence used to assess potential environmental effects of effluent discharges. When interpreting sublethal toxicity data it is important to keep in mind that laboratory toxicity test results may not accurately predict toxicity in receiving environments. Laboratory tests involve single species that may or may not be found in the study area, and there is an assumption that there is no background toxicity in the area. Furthermore, effluent plume dispersion and dilution vary due to tides and currents, and receiving waters and effluent quality characteristics vary seasonally or in other ways that could affect toxicity levels.

Sublethal effects of Howe Sound effluent on echinoderms and topsmelt were observed at average concentrations above 32.8%. Such concentrations are well above what has been observed in the receiving environment of HSLP and would not be expected to occur beyond the initial dilution zone surrounding the outfall. Sublethal effects on *Champia parvula* reproduction, however, were observed at average concentrations of 2.7%. Concentrations similar to this (2.3%) were observed during an effluent dispersion study conducted by Hodgins and Knoll in 1989.

Relative to previous EEM cycles, results of sublethal testing indicated improved effluent quality for echinoderm and topsmelt in Cycle Five, but decreasing quality for *Champia parvula* reproduction (Figure 3.6, Table 3.1).

Figure 3.6 IC25, EC25, or LC50 Geomean Endpoints for HSLP, EEM Cycle One through Cycle Five.



4.0 FISH POPULATION SURVEY

Summary of Fish Population Survey for HSLP EEM Cycle Five:

- An extended 28 day topsmelt sublethal toxicity test was performed for the HSLP EEM Cycle Five fish survey;
- High mortality rates of the control (36%) in exceedance of the maximum allowable rate (35%) were reached by Day 13 of the 28 day test. High mortality rates and inconsistent dose response were also associated with the majority of effluent test concentrations. Consequently, the test was terminated after day 13;
- Cantest was not able to identify the specific cause of the high mortality rates; however, they indicated there was likely an issue with the test organisms themselves or the test protocols. Sensitivity of organisms to handling, particularly during hatch, was identified as a potential reason for the test's failure;
- The HSLP LMC Committee, on advice from the EEM Science Committee, explored potential alternatives for completing the Cycle Five fish survey. However, both committees agreed that none of the proposed alternatives were suitable or feasible for implementation in Cycle Five; and
- An expanded benthic invertebrate survey as an alternative to the fish survey will be explored as a possible approach in Cycle Six.

4.1 INTRODUCTION

The fish survey for EEM is designed to provide an assessment of whether differences exist in fish characteristics between exposed and reference areas. The study design should be based on the ability to detect a difference in reproduction endpoints (relative gonad size) and fish health (liver size or condition) between reference and exposed areas (Environment Canada 2005). However, several confounding factors present in the Howe Sound marine environment limit or preclude the ability to perform an effective standard fish survey including:

- An absence of barriers to restrict finfish movement;
- Patchy distribution of non-finfish species (e.g., wild mussels);
- A lack of life history data available for most marine fish species; and
- Trapping of effluent at varying depths based on temperature, season, and currents, making it difficult to assess exposure.

In Cycle One, a standard fish survey was attempted in Howe Sound. It proved difficult to find suitable adult sentinel finfish species; difficulties were attributed to poor catch success, fish mobility, and uncertainty regarding exposure of fish related to depth and dispersion of effluent. As a result, in subsequent cycles alternative methods were explored, including a caged bivalve survey, a mesocosm survey, and an alternative extended sublethal toxicity test (Environment Canada 2005). The first alternative, caged bivalve surveys, was considered to be unsuitable for several reasons including:

1. Steep bathymetry of Howe Sound makes it difficult to deploy cages within the effluent zone and ensure that they will not be lost during the study period (as seen with pilot studies by BC MELP in 1997 and 1998);
2. Log booming is prevalent in Howe Sound and limits availability of suitable caging sites;
3. Influence of the Squamish River on the near-field zone and caged mussels;
4. A suitable tissue tracer was not available to confirm exposure to mill effluent, particularly important for HSLP where the effluent is trapped at depth; and
5. Logistical challenges related to deploying caged bivalves in an area of extensive commercial and recreational marine traffic.

A mesocosm study was also considered unsuitable for Howe Sound because of difficulties obtaining marine control/dilution water unaffected by mill effluent or the Squamish River, and problems identifying/collecting appropriate marine fish species to be used as a test species. In addition, refinements to the EEM mesocosm approach/apparatus are ongoing and its application to marine environments has been limited.

In EEM Cycle Three, an extended sublethal toxicity test (28-day topsmelt larvae growth and survival test) was determined to be the most suitable alternative to the standard fish survey. The objective of the test was to provide supplementary information about the potential effects of mill effluent on marine fishes and assess the potential of this alternative approach for future EEM programs. The results were inconclusive, however, given the reliability of the data were compromised by considerable variability in control survival rates.

In Cycle Four an Investigation of Cause Study was performed to evaluate effects on fish habitat, and consequently a fish population survey was not completed.

In Cycle Five, several fish surveys and alternatives were evaluated and considered, including standard adult finfish surveys, invertebrate shellfish surveys, and alternatives to fish surveys. By a process of elimination described in the design report (Hatfield 2009a), an extended topsmelt sublethal toxicity test was determined to be the most suitable alternative for the fish survey; the test was based on and used to verify the results of the Cycle Three test, with quality control measures improved. The test was discussed and agreed upon as the best approach for the Cycle Five fish survey by Al Strang (HSLP), Dr. Wade Gibbons (Hatfield), Janice Boyd (Environment Canada), Dr. Kelly Munkittrick (UNB, Science Advisor), and Dr. Simon Courtenay (DFO, Science Advisor), in a teleconference on April 2, 2008.

4.2 METHODS

4.2.1 Modifications to Sampling Design

The effluent dilution series proposed in the design document for Cycle Five was a repeat of the Cycle Three series of 1%, 2.5%, 6.4%, 16%, 40%, and 100% effluent. Unfortunately, the dilution series used by the laboratory for the test was the more routine 0.5-factor dilution series of 6.25%, 12.5%, 25%, 50%, and 100% effluent. Following discussions with Environment Canada, the test was not repeated using the correct dilution series for the following reasons:

- Correcting the dilution series would not have prevented the test from failing on day 13, given that the original test failed due to poor survivorship of embryos/larvae in the control treatment (i.e., independent of the dilution series).
- There was no evidence of a dose response among treatments using the 0.5-factor dilution series, and therefore it was likely no dose response would be observable even if the dilution series were corrected.

No other modifications were made to the Howe Sound EEM Cycle Five design (Hatfield 2009a).

4.2.2 Test Method

There is no standard test method recognized by Environment Canada or USEPA for an extended topsmelt (embryo-larvae) sublethal toxicity test. As in Cycle Three, the Cycle Five test method was based on a modified USEPA method for a seven-day topsmelt test and the Environment Canada method for early life stage rainbow trout test.

4.2.2.1 General Procedure

The 28-day topsmelt (*Atherinops affinis*) embryo-larvae sublethal test is a static renewal test that assesses the toxicity of a sample by comparing survival of early life stages (embryo and larvae) and growth of larvae for topsmelt exposed to effluent versus those in control water.

The general test procedure is based on information provided in the US EPA methodology document, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents on Receiving Waters to West Coast Marine and Estuarine Organisms" (US EPA 1995) and the chapter "Methods for Spawning, Culturing and Conducting Toxicity Tests with Early Life-Stages of Estuarine and Marine Fishes" (Middaugh *et al.* 1993). In addition, general testing procedures outlined in "Toxicity Testing Using Early Life Stages of Salmonid Fish (Rainbow Trout), July 1998, EPS 1/RM/28" (Environment Canada 1998) were used, where appropriate. Other publications by Anderson et al. (1991), Goodman et al. (1992), Middaugh and Anderson (1993) and Hunt et al. (submitted manuscript) provided supplementary information on methods for conducting early life stage bioassays using topsmelt.

4.2.2.2 Effluent Dilution Series

The effluent dilution series used for the extended topsmelt sublethal toxicity test was: 6.25%, 12.5%, 25%, 50%, and 100% effluent.

4.2.3 Sample Collection

4.2.3.1 Effluent

Mill personnel filled three 10-L sample containers with 100% final effluent and shipped them weekly to Cantest (Vancouver, BC) for the duration of the test, August 14 to August 27, 2009. Weekly shipments ensured fresh batches of effluent were provided to enhance quality throughout the testing process.

4.2.3.2 Embryos

Fertilized embryos were shipped from the supplier (Aquatic Biosystems, Fort Collins, CO) to Cantest (Burnaby, BC) the day they were spawned. After one day of acclimation, the eggs were two days old at the start of the test.

4.2.4 Test Conditions

The test was conducted in 600 mL glass beakers, and the volume in each test vessel was 200 mL. Vessels were covered with plexiglass sheets during the test to include the possible toxic effects of volatile compounds and to prevent the potential for contamination and evaporation. The test included five replicates per effluent concentration and five topsmelt per test vessel.

The control/dilution water was uncontaminated sand-filtered seawater from the Vancouver Aquarium (salinity of 29.8 to 31.1 ppt). If necessary, the salinity of effluent samples was adjusted to the desired range using artificial sea salts. Under these circumstances, an artificial sea salt control was included in the test.

The test was conducted in a constant temperature-controlled room of 20.0°C. The photoperiod was a 16:8 hour light:dark cycle (intensity of 10-20 µE/m²/s). During the test, the aeration of test solutions did not exceed 100 bubbles/minute delivered via airline tubing and a disposable Pasteur pipette. Once the embryos hatched, topsmelt larvae were fed newly hatched *Artemia nauplii* (25 µL) twice daily.

Embryo and larvae mortalities and deformities were observed and recorded, and dead fish were removed every 24 hours. Dissolved oxygen, pH, temperature and salinity were measured and recorded at the start and end of each 24-hour period in a representative vessel in each effluent concentration and controls.

4.2.5 Test Endpoints

The proposed biological endpoints for the extended topsmelt test included:

- Embryo survival and hatching success;
- Time to hatch;

- Incidence of larval deformity; and
- Larval survival and growth.

Percentages of viable embryos and larvae are calculated and used to determine the EC50/LC50 for both embryo and alevin survival using the maximum likelihood probit method. If no partial mortalities are observed or if the dose response is not linear, other methods (e.g., log-logit non-linear interpolation, Trimmed Spearman Karber method) can be used to analyze the data. EC25 and EC50 endpoints include 95% confidence intervals. The IC25 or IC50 values (with 95% CI) for growth are calculated using point estimation techniques, such as linear interpolation. NOEC and LOEC endpoints can be calculated for both embryo and larva survival, and larval growth using appropriate hypothesis testing (ToxCalc TM, Version 5.0).

As discussed in Section 4.2.1, it was not possible to calculate these endpoints because the test failed due to poor survivorship of embryos/larvae in the control treatment.

4.3 RESULTS AND DISCUSSION

4.3.1 Failed Test Results

Cantest initiated an early life stage test using fertilized topsmelt (*Atherinops affinis*) eggs which were exposed to varying concentrations of HSLP mill effluent during embryo development, hatching, and larval development. The eggs hatched between day seven and 11 of the test, at which point feeding of *Artemia* (brine shrimp) commenced.

Thirteen days into the 28 day static renewal test, the combined mortality rate of the salt controls was 36.0% (Table 4.1), which exceeded the maximum allowable rate stipulated in the protocols (35%). High mortality rates were also associated with the majority of effluent test concentrations without a defined dose response, which indicated an issue with the test organisms themselves or the test protocols. As a result of the high mortality rates, the test was terminated, with approval from Environment Canada (J. Boyd, Environment Canada, *pers. comm.*, August 29, 2009). Given the questionable nature of the data due to the high control mortality, statistics were not performed on the test results.

Cantest was not able to identify what caused the high mortality rates in the extended sublethal toxicity test. However, it was known prior to performing the test that topsmelt are sensitive organisms, and that despite taking several precautions their survival under the applied test conditions would be very challenging. It is for these reasons that extended topsmelt tests are not routinely performed by ecotoxicology labs. Handling and sensitivity during hatch were proposed as possible reasons for the test's failure. However, Cantest was confident they had applied all possible precautions and QA/QC measures to minimize handling issues, and noted that any further issues associated with handling would be difficult to address.

Further details regarding testing procedures and its results are provided in a full report produced by Cantest in Appendix A2.

4.3.2 Alternatives Considered

The LMC re-convened following the test failure to discuss alternatives for completing the Cycle Five HSLP EEM fish survey program. Members of the EEM Science Committee (i.e., Simon Courtenay, Fisheries and Oceans; Kelly Munkittrick, University of New Brunswick; and Mark McMaster, Environment Canada) proposed retrying the extended topsmelt test; however, Cantest advised against repeating the test, given the history of its failure in general, at other mills during previous cycles, and in Cycle Five for HSLP, as well as the general knowledge among ecotoxicologists that marine fish egg tests are unreliable. Instead, Cantest suggested a number of laboratory bioassays that might be applied to complete the program in place of the extended topsmelt sublethal toxicity test, including:

- Bivalve larval development test: A reliable, but sensitive, EPA standard 48-hour test involving fertilized gametes and seawater control; endpoints include normality (i.e., normal vs. abnormal development) and survival.
- Echinoderm larval development test: A less reliable, EPA standard 48-hour test involving sand dollars or sea urchins and seawater control; endpoints include normality and survival.
- Rainbow trout egg-alevin test: A reliable, EPA standard test using freshwater rainbow trout embryos/alevins, that ends seven days after 50% of control eggs hatch (approx. 30 days); endpoints include normality and survival.

The LMC, with additional input from EEM Science Committee members, decided that none of the proposed alternatives would be suitable or reliable for completing the Cycle Five EEM fish survey objectives (J. Boyd, Environment Canada, *pers. comm.*, September 23, 2009). The primary concern was that the substitute laboratory bioassays would not adequately answer the EEM fish survey question regarding potential effects of mill effluent on local fish populations. The bivalve and echinoderm larval tests would have provided similar results to the standard EEM 48-hour echinoderm fertilization test performed twice monthly for the sublethal toxicity program. The rainbow trout egg-alevin test, although longer than 48 hours, uses a freshwater test species, which is unlikely to provide data that is relevant to the marine environment.

Given the nature of the receiving environment in Howe Sound and the difficulties associated with directly evaluating effects of effluent on fish populations in the area, the LMC also reviewed EEM Science Committee members' recommendation for performing an expanded benthic invertebrate survey study as a fish study alternative. Both committees agreed there was insufficient time remaining in Cycle Five to adequately implement such a comprehensive study. Furthermore, it was not considered necessary to perform such a study to meet regulatory requirements for Cycle Five (J. Boyd, Environment Canada, *pers. comm.*, September 30, 2009), given that the failed extended topsmelt test still would provide reportable (although unsuccessful) results and an expanded benthic invertebrate study was recently conducted in Cycle Four.

4.4 RECOMMENDATIONS

During the current program, the EEM Science Committee suggested an expanded benthic survey as an alternative to the fish survey. Given that HSLP will be required to conduct a benthic invertebrate survey as part of their routine Cycle Six EEM program, the LMC Committee has recommended that an expanded version be considered during the next design phase.

Table 4.1 Results of the extended topsmelt sublethal toxicity test (Day 1 to 13), Howe Sound Pulp and Paper Limited Partnership Cycle Five EEM, 2009.

		# Surviving Organisms																
Conc. (%v/v)	Rep #	Day/Date													% Survival	% Mean Survival	% Mean Mortality	SD (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13				
		08/15	08/16	08/17	08/18	08/19	08/20	08/21	08/22	08/23	08/24	08/25	08/26	08/27				
Sea Water	A	5	5	5	5	5	5	5	5	5	5	4	4	4	80%	72%	28%	23%
Control	B	5	5	5	5	5	4	4	3	3	2	2	2	2	40%			
	C	5	5	5	5	5	5	5	4	4	3	3	3	3	60%			
	D	5	5	5	5	5	5	5	5	5	4	4	4	4	80%			
	E	5	5	5	5	5	5	5	5	5	5	5	5	5	100%			
Salt	A	5	5	5	5	5	5	5	5	5	4	4	4	1	20%	56%	44%	22%
Control	B	5	5	5	5	5	5	5	5	5	2	3	3	3	60%			
	C	5	4	4	4	4	4	4	4	4	3	3	3	3	60%			
	D	5	5	5	5	5	5	5	5	5	4	4	4	4	80%			
	E	5	5	5	5	5	5	5	3	3	3	3	3	3	60%			
6.25	A	5	5	5	5	5	5	5	5	5	4	3	3	3	60%	76%	24%	17%
	B	5	5	5	5	5	5	5	5	5	4	4	4	4	80%			
	C	5	5	5	5	5	5	5	5	5	5	3	3	3	60%			
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	100%			
	E	5	5	5	5	5	5	5	5	5	5	5	5	4	80%			
12.5	A	5	5	5	5	5	5	5	4	3	3	2	2	2	40%	40%	60%	14%
	B	5	5	5	5	5	5	5	4	4	4	4	2	2	40%			
	C	5	5	5	5	5	3	3	3	3	3	3	2	2	40%			
	D	5	5	5	5	5	5	5	5	5	5	1	1	1	20%			
	E	5	5	5	5	5	5	5	5	5	4	3	3	3	60%			
25	A	5	5	5	5	5	5	5	4	4	4	3	1	0	0%	44%	56%	46%
	B	5	5	5	5	5	5	5	3	3	3	3	0	0	0%			
	C	5	5	5	5	5	5	5	5	5	5	4	4	4	80%			
	D	5	5	5	4	4	4	4	3	3	3	2	2	2	40%			
	E	5	5	5	5	5	5	5	5	5	5	5	5	5	100%			
50	A	5	5	5	5	5	5	5	5	4	3	3	3	3	60%	52%	48%	30%
	B	5	5	5	5	5	5	5	4	2	2	0	0	0	0%			
	C	5	5	5	5	5	5	5	4	4	4	4	4	3	60%			
	D	5	5	5	5	5	5	5	5	4	4	4	4	3	60%			
	E	5	5	5	5	5	5	5	5	5	4	4	4	4	80%			
100	A	5	5	5	5	5	5	3	2	2	2	2	2	1	20%	40%	60%	32%
	B	5	5	5	5	5	5	5	5	2	2	2	2	2	40%			
	C	5	5	5	5	5	5	5	5	5	5	0	0	0	0%			
	D	5	5	5	5	5	5	5	5	5	5	3	3	3	60%			
	E	5	5	5	5	5	5	5	5	5	5	4	4	4	80%			

5.0 FISH TISSUE SURVEY

Summary of Fish Tissue Survey for HSLP EEM Cycle Five:

- Chlorinated dioxins and furans were monitored in sediments, crab, and finfish within the receiving environment during Cycle Five as required by Health Canada and Environment Canada (i.e., separate from EEM); results of the 2009 monitoring program are summarized herein;
- In 2009, total TEQ concentrations in crabs at three stations were above the Health Canada consumption threshold (24.4 pg/g hepatopancreas); levels at the remaining five stations were below the threshold. Total TEQ concentrations in dogfish liver (1041 pg/g) were over 34 times greater than the consumption threshold (30.0 pg/g liver), and the concentration in liver from a single English sole was below the threshold, measuring 13.8 pg/g; and
- No complaints regarding fish flavour or odour related to Howe Sound mill effluent were received in Cycle Five.

Under the federal EEM guidelines (Environment Canada 2005) mills which have used chlorine bleaching may be required to conduct analysis of tissue levels of chlorinated dioxin and furan congeners on edible portions of fish if dioxins and furans are an issue for the receiving environment. Mills are required to perform fish tissue analysis for dioxins and furans in two instances:

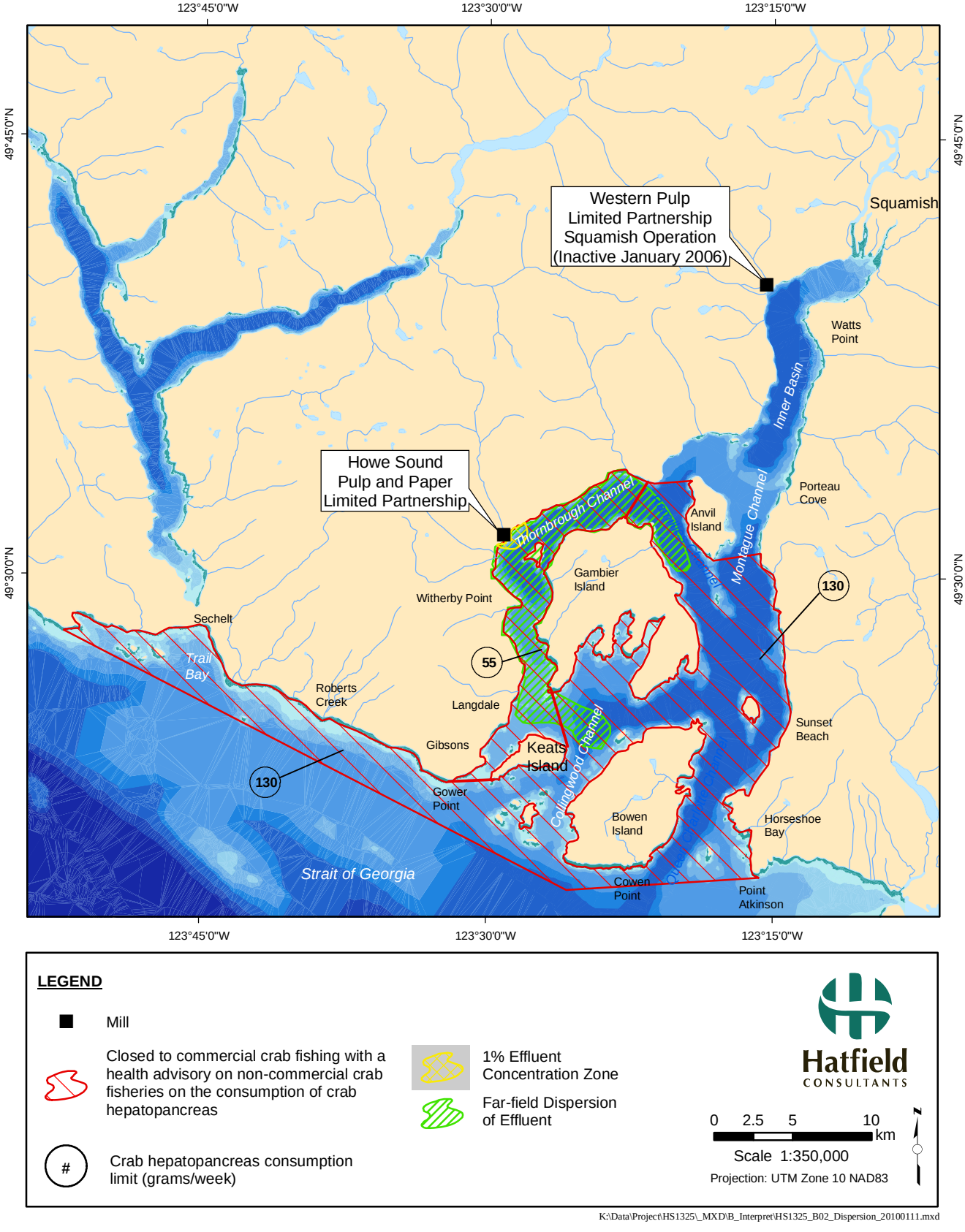
- Where mill effluent has contained measurable concentrations of 2,3,7,8-T_4CDD or 2,3,7,8-T_4CDF since the submission of the most recent interpretive report. The definition of measurable is provided in the *Pulp and Paper Mill Effluent Chlorinated Dioxins and Furans Regulation* (SOR/2000-102, s. 8) and *Reference Method for the Determination of PCDDs and PCDFs in Pulp and Paper Mill Effluents* (EPS 1/RM/19); and
- Where an effect on fish tissue was reported in the most recent interpretive report; an effect is defined as a concentration which exceeds 15 pg/g wet weight in muscle, 30 pg/g wet weight in finfish liver, and 24.4 pg/g wet weight in crab hepatopancreas.

As of the May 2004 *PPER* amendments, EEM no longer triggers fish tainting surveys, and only requires reporting of fish flavour or odour complaints (Sch IV.1 para. 12 (1)(i)).

5.1 TISSUE ANALYSES: DIOXINS AND FURANS

Based on ongoing recreational consumption advisories in Howe Sound (Figure 5.1), the HSLP mill was required to monitor dioxins and furans in crab, finfish, and sediments during the Cycle Five EEM Program, as per Government of Canada Directives. Detailed results and analyses for the 2009 HSLP dioxin/furan trend monitoring program are presented in Hatfield (2009b); a summary is presented below.

Figure 5.1 Estimated zones of effluent dispersion near Howe Sound Pulp and Paper Limited Partnership and 2009 fisheries closures in Thornbrough Channel.



LEGEND

- Mill
- Closed to commercial crab fishing with a health advisory on non-commercial crab fisheries on the consumption of crab hepatopancreas
- # Crab hepatopancreas consumption limit (grams/week)
- 1% Effluent Concentration Zone
- Far-field Dispersion of Effluent

Hatfield CONSULTANTS

0 2.5 5 10 km

Scale 1:350,000

Projection: UTM Zone 10 NAD83

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5.1.1 Sediment

Federal directives required sediment sampling at station S14, near the HSLP diffuser. Loss on ignition (LOI), an indicator of organic content, has fluctuated since monitoring began at the station in 1989 and has averaged 14%. In 2009, LOI was 13.6% at S14 and sediments in this area were predominantly comprised of sand (55%) and mud (44%). From the late 1980s to early 1990s, while elemental chlorine bleaching was being phased out at the mill, organochlorine concentrations in sediments at station S14 decreased annually (Figure 5.2). Since 1992, despite the complete elimination of elemental chlorine in 1997, concentrations of dioxins and furans have persisted in sediments near the diffuser, have not exhibited any patterns over time, and appear to be fluctuating at low levels. In 2009 this trend continued, and concentrations of 2,3,7,8-T_4CDD, 2,3,7,8-T_4CDF, and Total 2,3,7,8-T_4CDD Toxic Equivalency (Total TEQ) were within the lower end of the historical range (3.68 pg/g, 100 pg/g, and 36.4 pg/g, respectively).

5.1.2 Crab Hepatopancreas

Federal directives required crab collections at eight stations in Howe Sound: C9 (McNab Point), C10 (Port Mellon Outfall), C14 (West Keats Island), C16a (Port Graves), C19a (Cates Bay), C21 (S. Gower Pt.), C27 (Douglas Bay), and C28 (Brigade Bay). Required numbers of adult male Dungeness crab (n=7) were captured at stations C9, C10, C16a, and C21. Target numbers of crab could not be collected at the other four stations: five crabs were caught at C14, two crabs at C27, and one crab at C19a. No crabs were caught at station C28.

In 2009, concentrations of 2,3,7,8-T_4CDD in crab hepatopancreas ranged from 0.93 pg/g (C27) to 5.61 pg/g (C19a). 2009 concentrations of 2,3,7,8-T_4CDD in crabs at five of the seven stations measured (C9, C10, C16a, C19a, C27) were marginally higher than concentrations measured during the previous sampling program (Figure 5.3). Overall, however, results in 2009 were comparable to those reported in recent years, and considerably lower than concentrations reported in the late 1980s and early 1990s, when concentrations were as high as 300 pg/g (C10).

In 2009, concentrations of 2,3,7,8-T_4CDF in crab hepatopancreas ranged between 26.2 pg/g (C21) and 233 pg/g (C19a). Similar to dioxins, concentrations of 2,3,7,8-T_4CDF in crabs at five of the seven stations measured (C10, C16a, C19a, C21, C27) were marginally higher than concentrations measured during the previous sampling program (Figure 5.4). As with dioxins, results for furans in 2009 were within the lower range of concentrations observed historically and were considerably lower than concentrations reported in the late 1980s and early 1990s, when concentrations were as high as 9,100 pg/g (C10).

Crab total TEQ concentrations in 2009, ordered from largest to smallest, were: 86.0 pg/g at C19a, 43.6 pg/g at C9, 34.9 pg/g at C10, 23.9 pg/g at C14, 18.4 pg/g at C16a, 15.1 pg/g at C27, and 10.2 pg/g at C21. TEQ concentrations in crab hepatopancreas from three Howe Sound stations exceeded Health Canada's consumption advisory threshold of 24.4 pg/g TEQ: C19a (by 3.5%), C9 (by 1.8%),

and C10 (by 1.4%). TEQ concentrations at all other stations were below the threshold (Figure 5.5). At station C9, historical TEQ concentrations only dropped below 24.4 pg/g once when they dipped down to 12.7 pg/g in 2003. At stations C10 and C19a, concentrations in the past decade have fluctuated, falling below the threshold in some years and above the threshold in others.

Total TEQ values have dropped considerably since the onset of organochlorine monitoring at the mill; concentrations at all stations underwent an initial drop following pollution abatement changes at the mill in the early 1990s, followed by another drop in the early 2000s due to ongoing mill updates and changes in production levels (Figure 5.5). Since 2000, levels at most stations have continued to fluctuate from year to year within a lower concentration range (Figure 5.5). At three stations (C9, C14, C21), 2009 TEQ levels in crabs were higher than in the previous year measured. At four stations (C10, C16a, C19a, C27), 2009 concentrations were lower.

5.1.3 Finfish

Federal directives required finfish collections at station T9, in Queen Charlotte Channel, nearby crab sampling stations C27 and C28. The required numbers (n=7) of dogfish greater than 600 mm in length were captured at the station; a composite of all seven dogfish was submitted for analyses. The minimum target of one sole was also captured at the station.

In 2009, the concentration of 2,3,7,8-T_4CDD in composited dogfish liver was 218 pg/g; the concentration in dogfish muscle was over one hundred times lower (2.03 pg/g). This difference is consistent with past analyses in Howe Sound (e.g., Hatfield 2005), and exists due to the detoxifying role of the liver. The 2009 dioxin concentration in dogfish liver were higher than concentrations measured in recent years (2002 to 2005), but fell below concentrations measured prior to 2002 (Figure 5.6). In 2009, 2,3,7,8-T_4CDD in sole liver was low (2.3 pg/g) relative to dogfish liver (218 pg/g). The 2009 dioxin concentration in sole liver was among the lowest values measured historically in bottomfish at station T9, and was the lowest concentration measured in sole species (Figure 5.6).

In 2009, the concentration of 2,3,7,8-T_4CDF in composited dogfish liver was 4900 pg/g; the concentration in dogfish muscle was over 250 times lower (19.2 pg/g), a difference that is consistent with those seen in dioxins. Similar to dioxin concentrations, the 2009 furan concentration in dogfish liver was higher than concentrations measured in recent years (2002 to 2005), but fell below concentrations measured prior to 2002 (Figure 5.7). In 2009, 2,3,7,8-T_4CDF in sole liver was low (48.6 pg/g) relative to dogfish liver (4900 pg/g). The 2009 furan concentration in sole liver was among the lowest values measured historically in bottomfish at station T9, and was the lowest concentration to date in any sole species (Figure 5.7).

In 2009, total TEQ concentrations was highest in dogfish liver (1,041 pg/g), and significantly lower in dogfish muscle tissue (6.7 pg/g) and sole liver (13.85 pg/g). Overall, total TEQ concentrations in dogfish have decreased since

monitoring began in the late 1990s; however, concentrations have risen again since 2004 when they reached a low of 2.8 pg/g (Figure 5.8). TEQ concentrations in bottomfish have fluctuated since 1990. In general, however, TEQ levels in sole species have shown an overall decrease over time. Specifically, English sole TEQ in 1990 was 69.8 pg/g, and during the 2009 program it measured 13.8 pg/g.

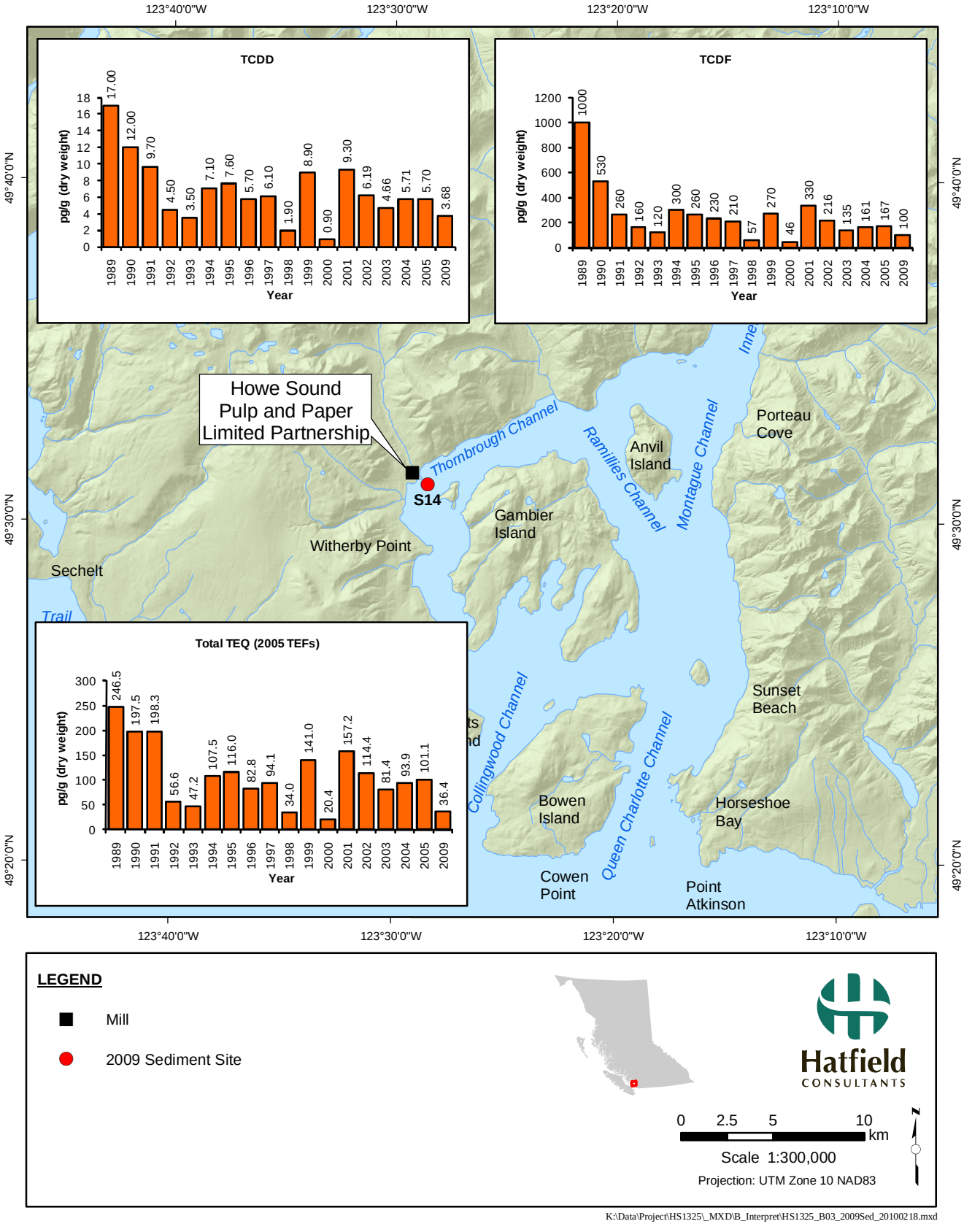
Historically, TEQ concentrations in dogfish livers collected from station T9 have exceeded Health Canada's consumption advisory threshold of 30.0 pg/g TEQ, with the exception of 2004 when TEQ levels dropped to 2.8 pg/g (Figure 5.8). In 2009, concentrations in dogfish liver (1041 pg/g) were over 34 times greater than the threshold.

Until 2004, bottomfish TEQ concentrations were generally above the consumption advisory threshold, ranging between 28.8 pg/g (black cod) and 75.3 pg/g (slender sole). In 2009, TEQ in the liver from a single English sole was below the consumption threshold, measuring 13.8 pg/g (Figure 5.8).

5.2 FISH FLAVOUR AND ODOUR COMPLAINTS

No reports of fish flavour or odour related to Howe Sound effluent were received by the mill during Cycle Five.

Figure 5.2 Concentrations of 2,3,7,8-T4CDD, T4CDF and Total TEQ in sediments collected from Howe Sound, 1989 to 2009.



LEGEND

- Mill
- 2009 Sediment Site

Hatfield CONSULTANTS

0 2.5 5 10 km

Scale 1:300,000

Projection: UTM Zone 10 NAD83

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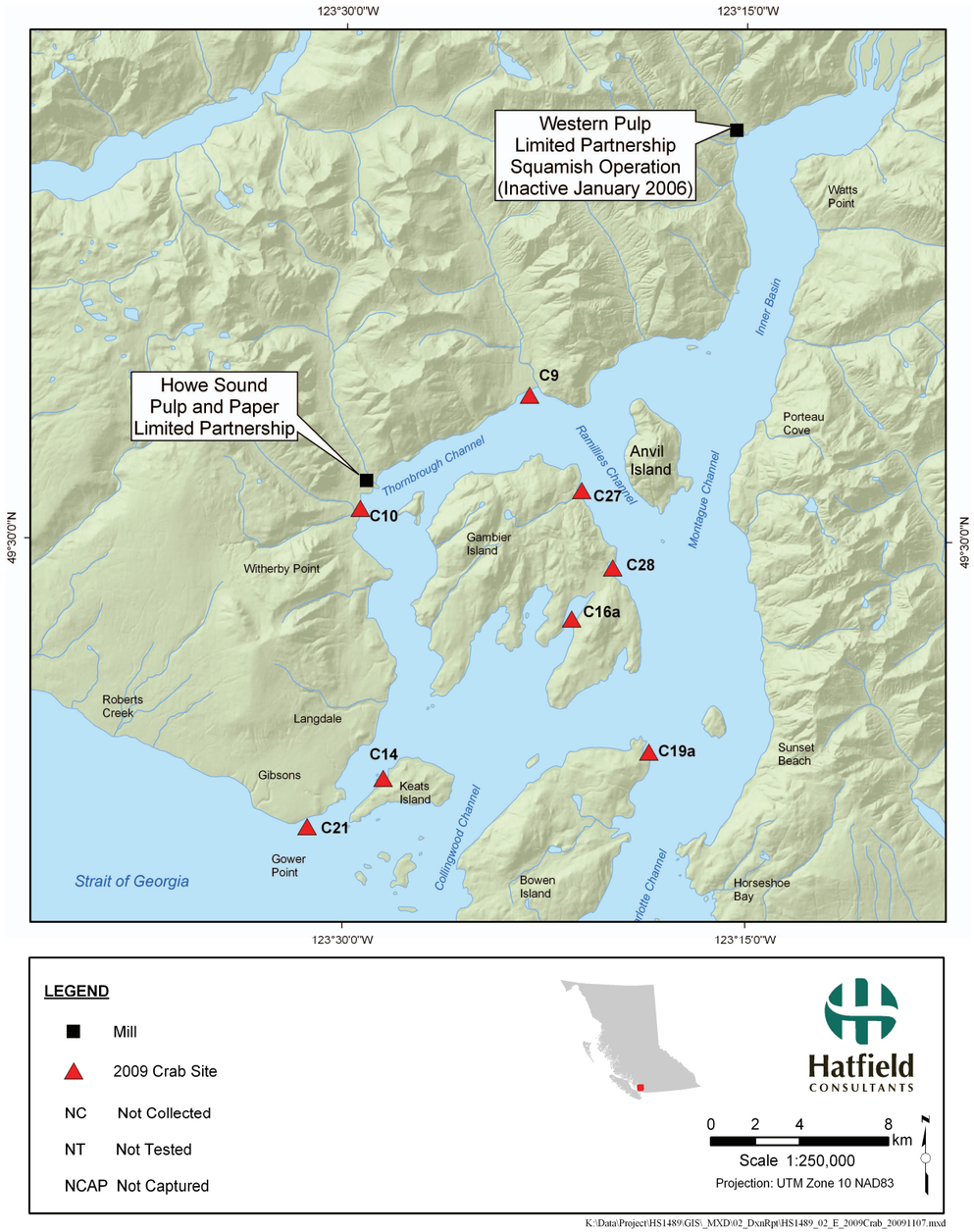
Figure 5.3 Concentrations of 2,3,7,8-T_4CDD in Dungeness crab hepatopancreas collected from Howe Sound, 1989 to 2009.



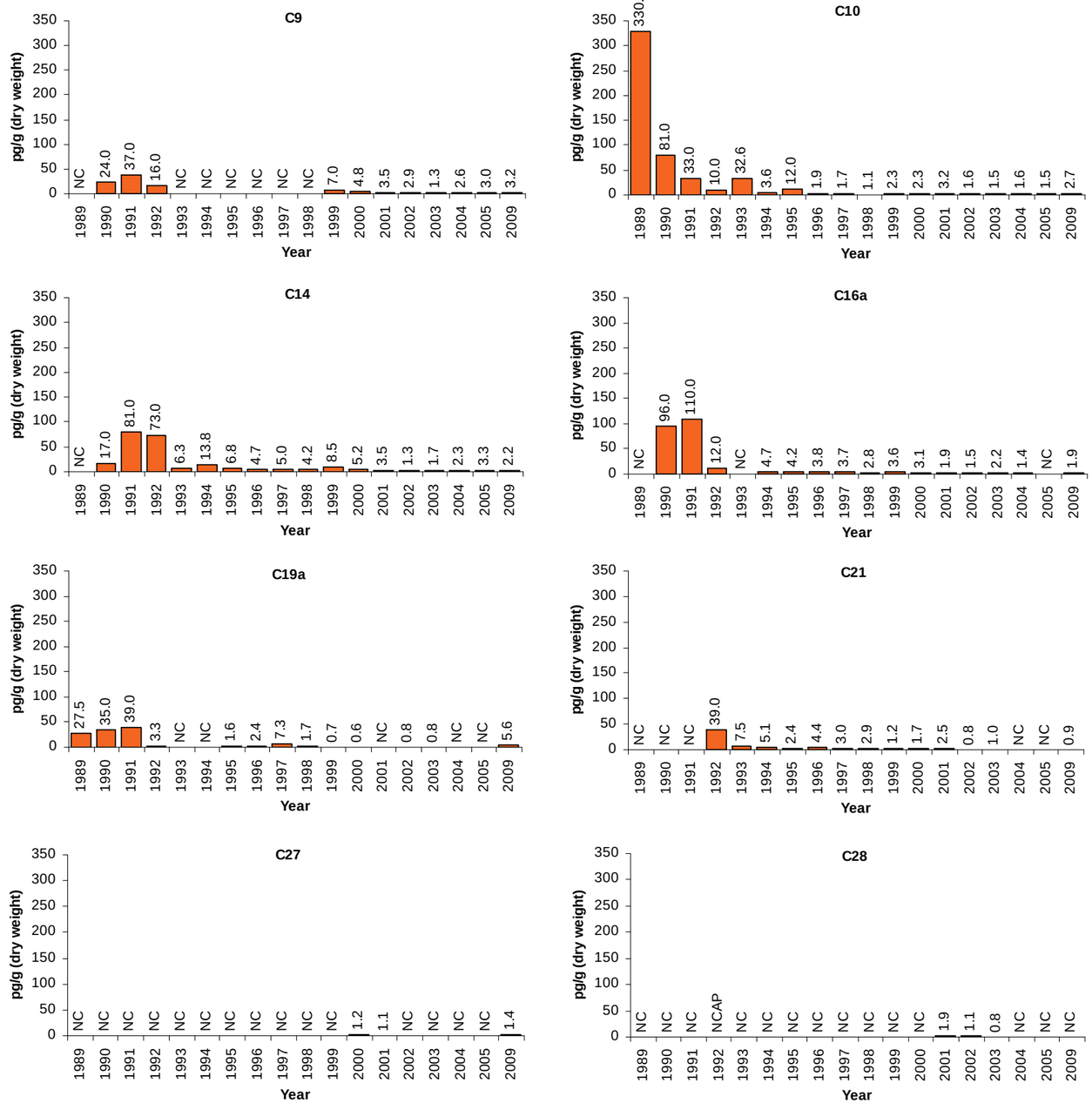
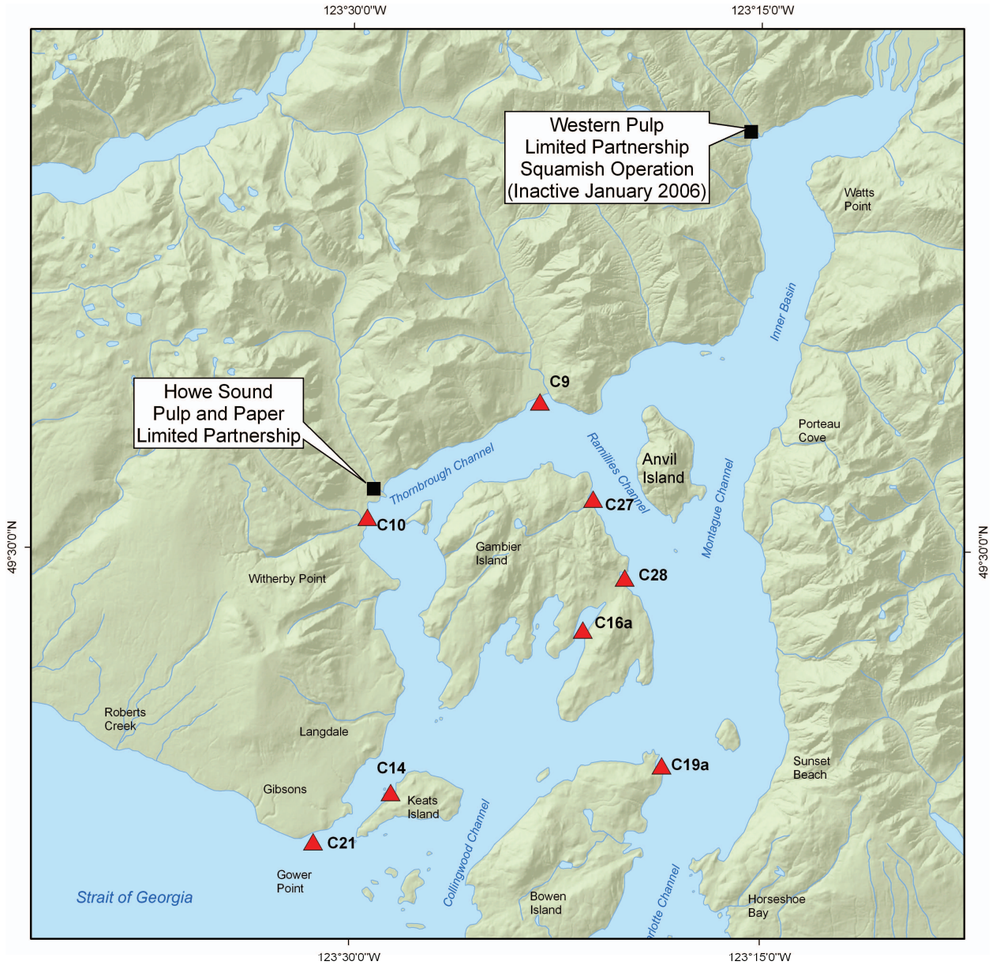
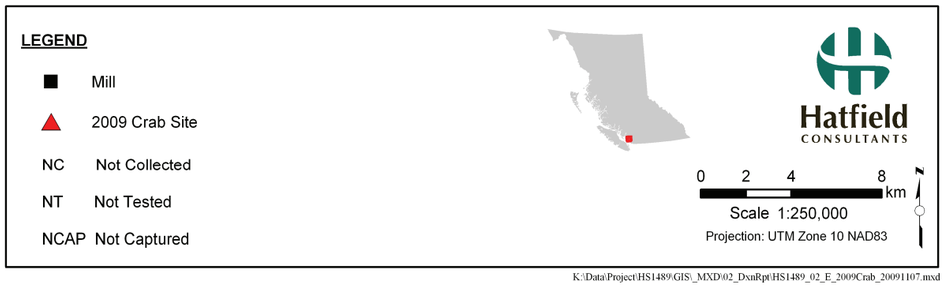


Figure 5.4 Concentrations of 2,3,7,8-T_4CDF in Dungeness crab hepatopancreas collected from Howe Sound, 1989 to 2009.





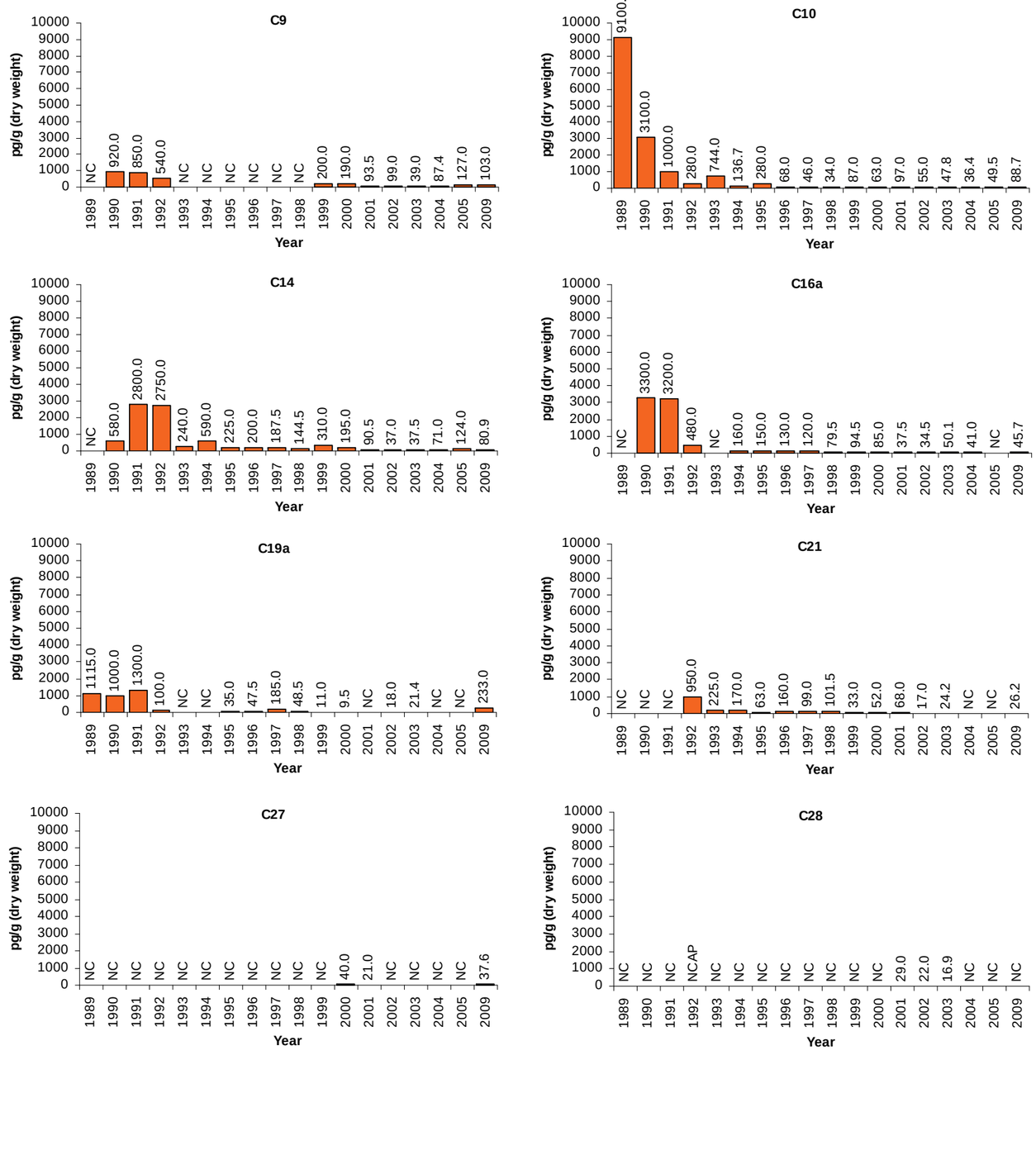
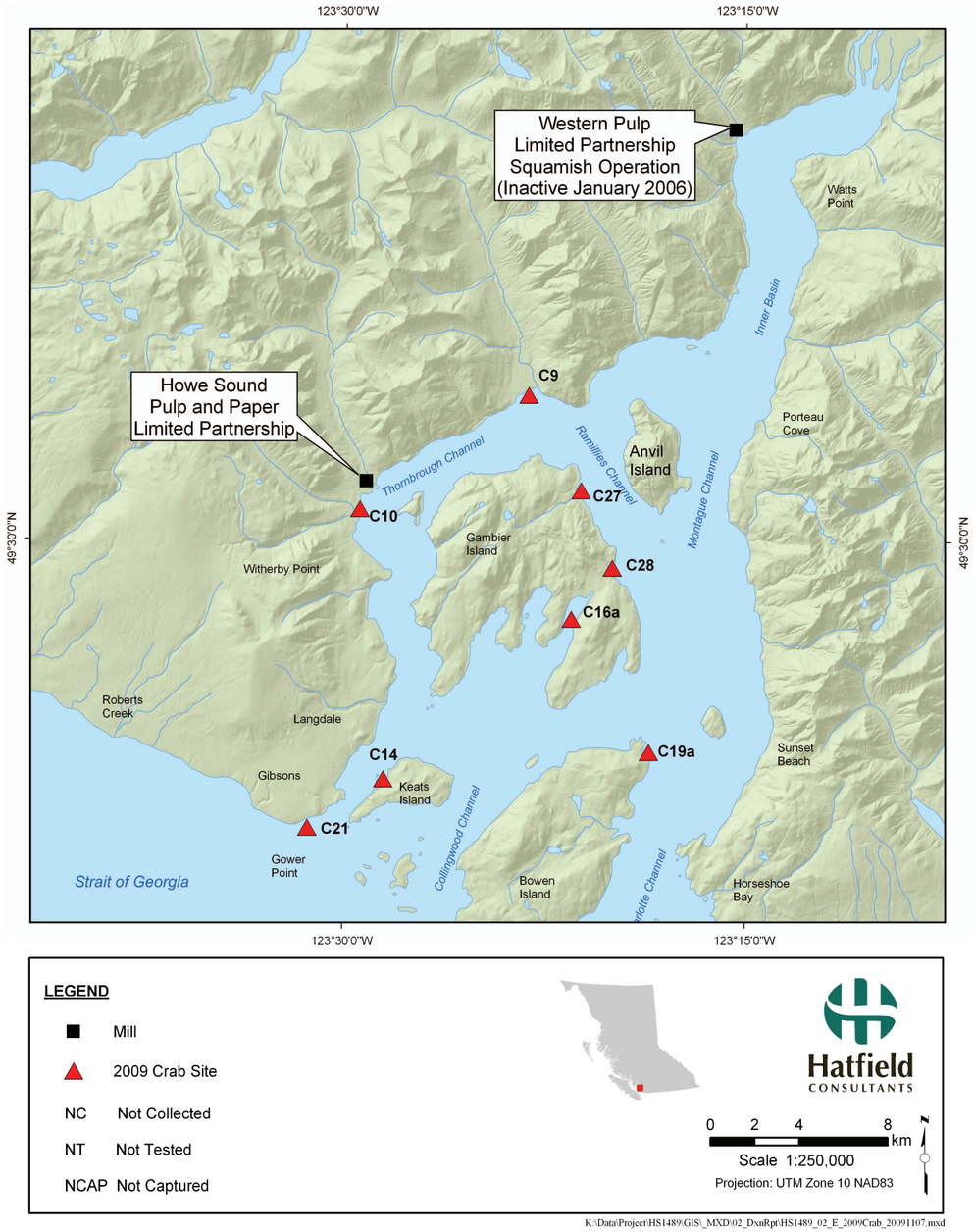


Figure 5.5 Concentrations of 2,3,7,8-T_4CDD TEQ in Dungeness crab hepatopancreas collected from Howe Sound, 1989 to 2009.



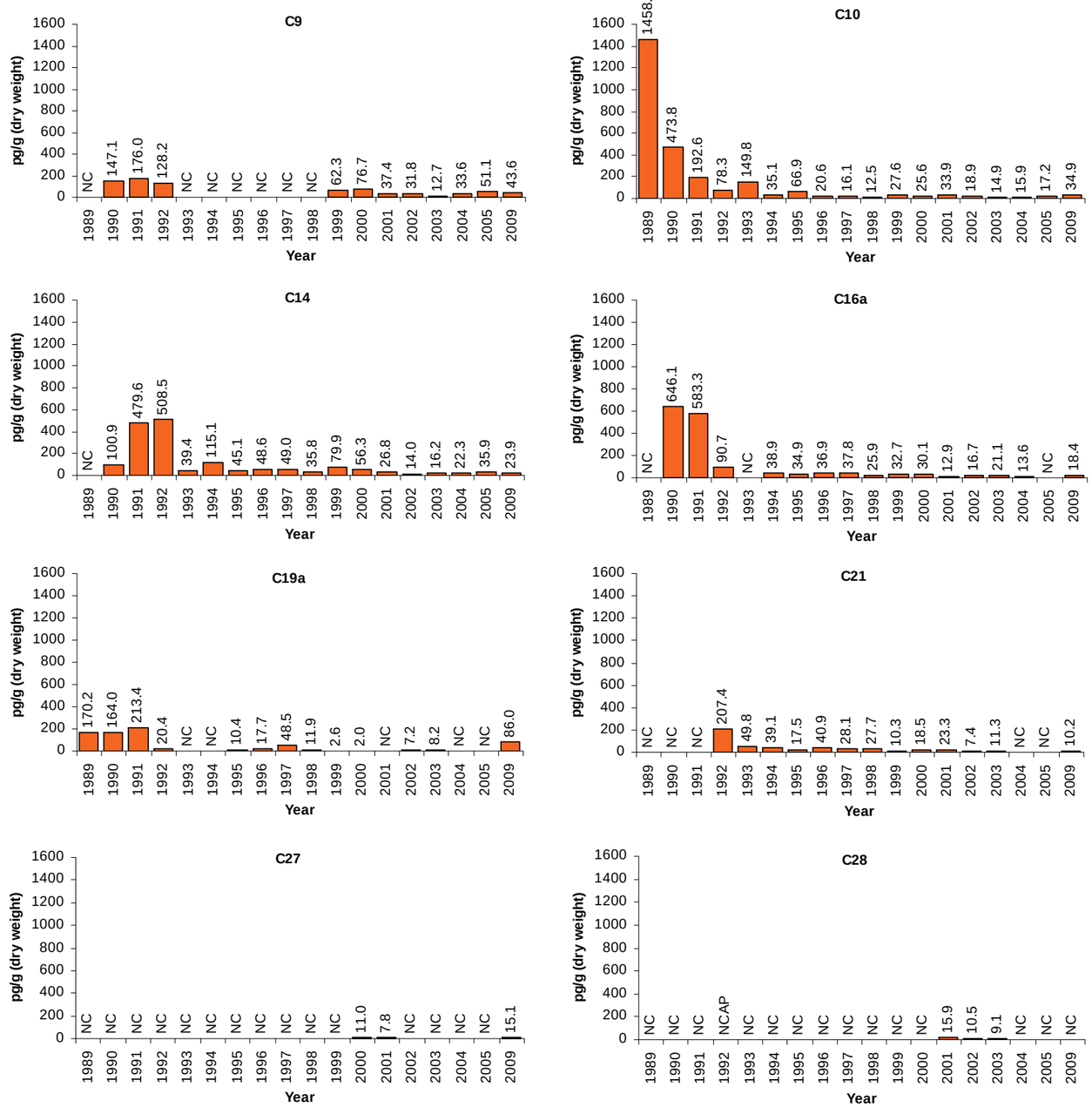
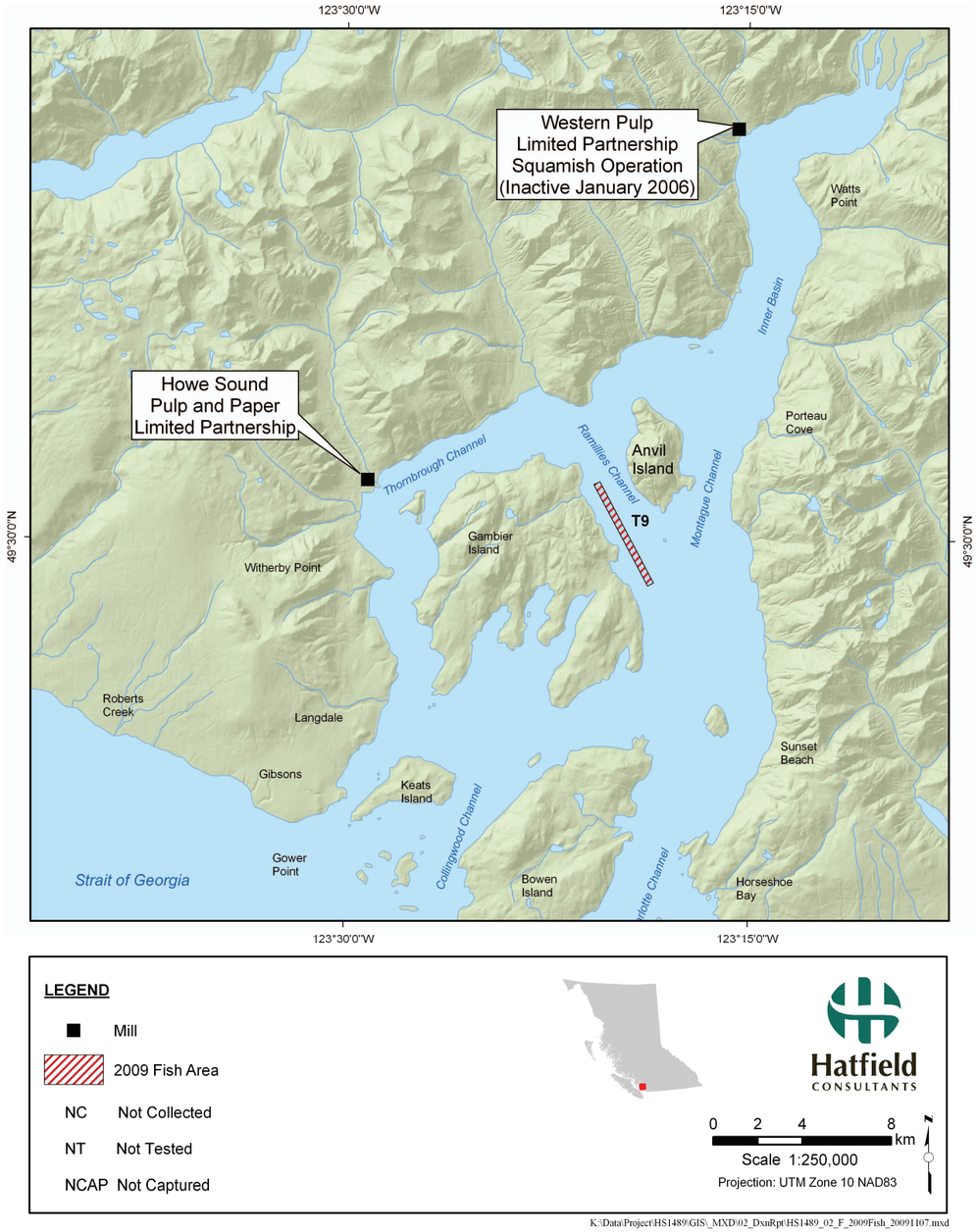
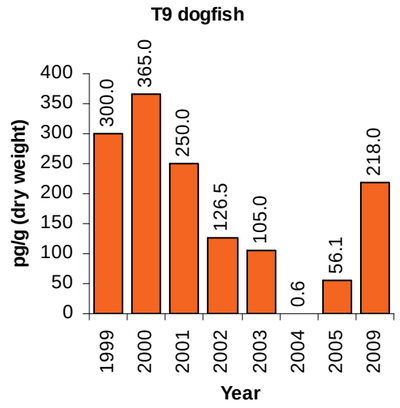


Figure 5.6 Concentrations of 2,3,7,8-T_4CDD in dogfish and sole liver collected from Howe Sound, 1989 to 2009.





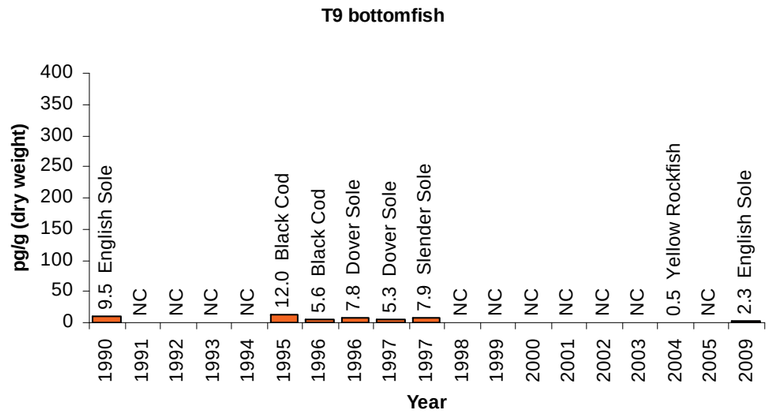
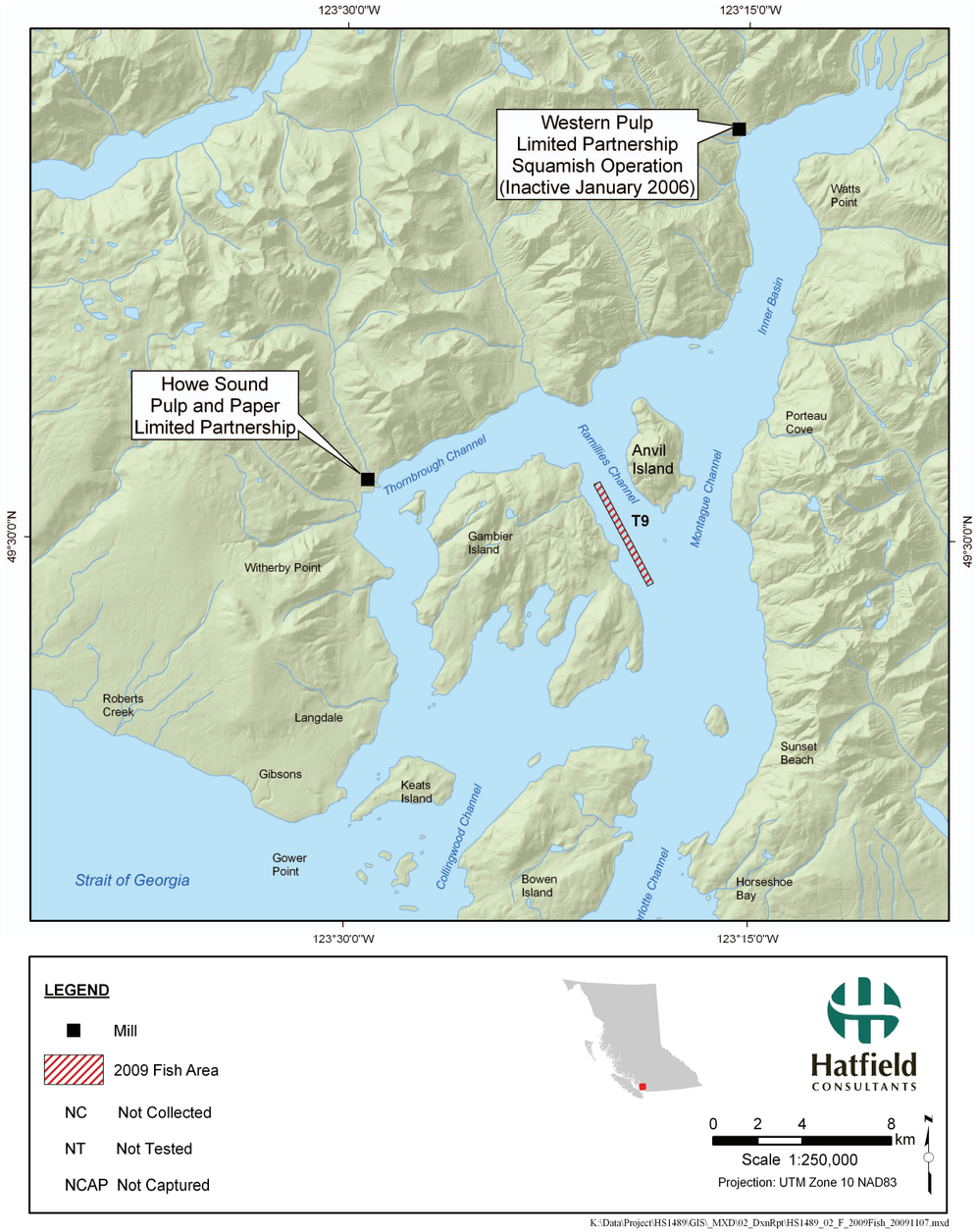
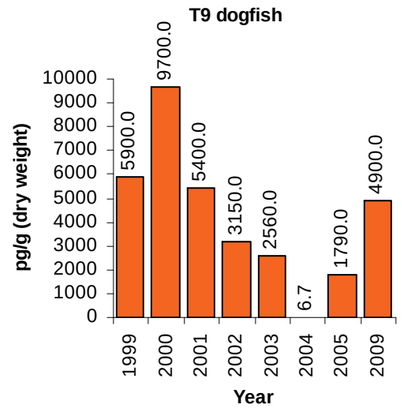


Figure 5.7 Concentrations of 2,3,7,8-T_4CDF in dogfish and sole liver collected from Howe Sound, 1989 to 2009.





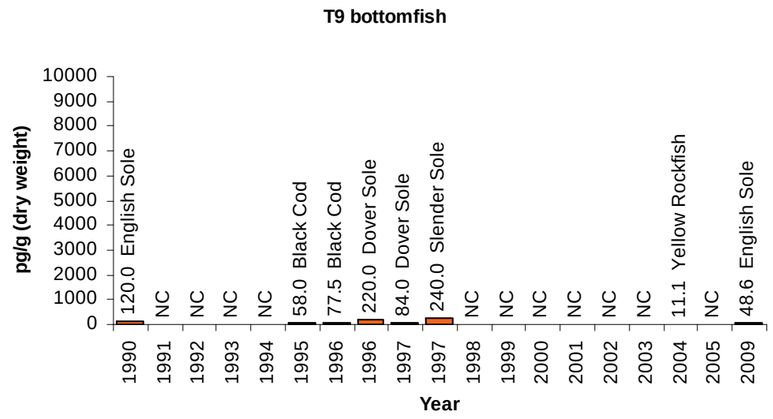
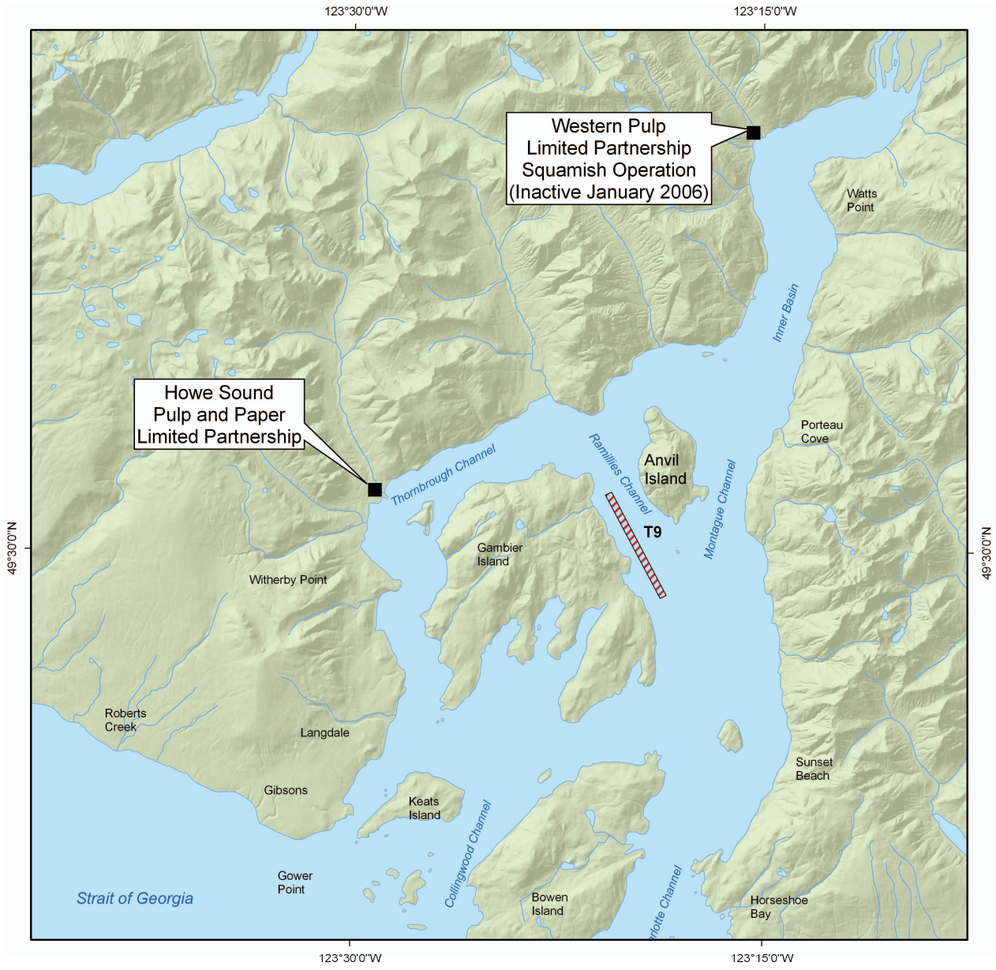
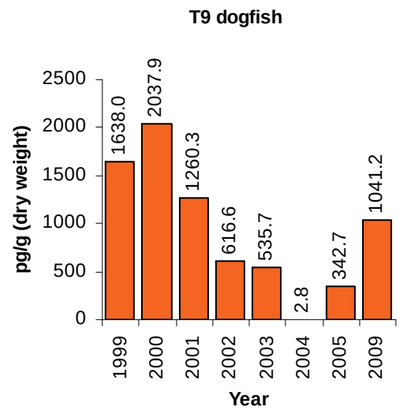
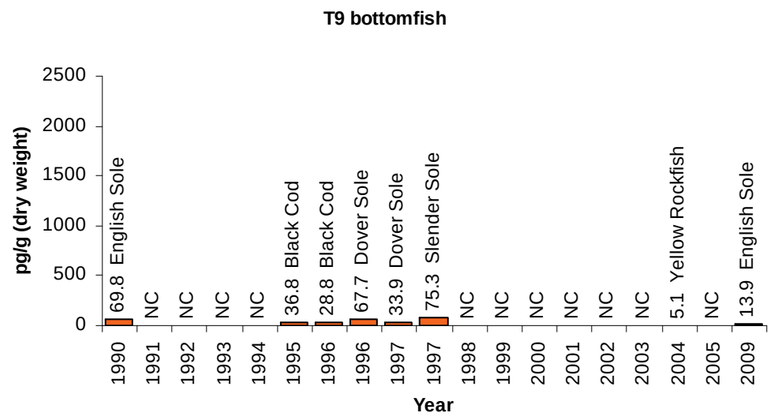


Figure 5.8 Concentrations of 2,3,7,8-T_4CDD TEQ in dogfish and sole liver collected from Howe Sound, 1989 to 2009.







6.0 CONCLUSIONS

Based on the results of the Howe Sound EEM Cycle Five program, the following conclusions can be made:

- No effect of effluent on topsmelt (*Atherinops affinis*) larvae growth (IC25 endpoint) and survival (LC50 endpoint) was observed.
- Effects on echinoderm fertilization were observed at a mean effluent concentration of 32.8% (IC25).
- *Champia parvula* reproduction was affected at a mean effluent concentration of 2.7% (IC25).
- Based on a 1% effluent concentration zone of 2,000 m from the diffuser, maximum potential zones of sublethal effect from the effluent discharge point were <20 m for fish growth and survival, 61 m for invertebrate fertilization, and 743 m for algal reproduction.
- During the 2009 dioxin/furan trend monitoring program, total TEQ concentrations in crabs at three stations were above Health Canada's consumption threshold (24.4 pg/g hepatopancreas); levels at the remaining five stations were below the threshold.
- Total TEQ concentrations in dogfish liver were over 34 times greater than the threshold (30.0 pg/g liver), and the concentration in liver from a single English sole was below the consumption threshold.
- An extended 28-day topsmelt sublethal toxicity test was performed for the HSLP EEM Cycle Five fish survey, with the following outcomes:
    - High mortality rates of the control (36%) in exceedance of the maximum allowable rate (35%) were reached by Day 13 of the 28 day test.
    - High mortality rates and inconsistent dose response were also associated with the majority of effluent test concentrations.
    - Due to mortality rates for both the control and effluent test concentrations, the test was terminated after Day 13.
    - Cantest could not identify the specific cause of the high mortality rates; however, they indicated there was likely an issue with the test organisms themselves or the test protocols. Sensitivity of organisms to handling, particularly during hatch, was identified as a potential reason for the test's failure.
    - The HSLP LMC Committee, on advice from EEM Science Committee members, explored some potential alternatives for completing the Cycle Five fish survey. However, both committees agreed that none of the proposed alternatives were suitable or feasible for implementation in Cycle Five.
    - An expanded benthic invertebrate survey will be explored as a possible alternative approach to the fish survey in Cycle Six.

7.0 CLOSURE

We trust the above information meets your requirements. If you have any questions or comments, please contact the undersigned.

HATFIELD CONSULTANTS:

Approved by: _____ March 23, 2010

Nara Henderson, M.Sc. Date
Project Manager

Approved by: _____ March 23, 2010

Dr. Wade Gibbons, RPBio Date
Project Director

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9.0 GLOSSARY

Acute — With reference to toxicity tests with fish, usually means an effect that happens within four to seven days, or an exposure of that duration. An acute effect could be mild or sublethal, if it were rapid.

Benthos — Organisms that inhabit the bottom substrates (sediments, debris, logs, macrophytes) of aquatic habitats for at least part of their life cycle. The term benthic is used as an adjective, as in benthic invertebrates.

BOD — Biochemical oxygen demand. The test measures the oxygen utilized during a specified incubation period for the biochemical degradation of organic material and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. Usually conducted as a five-day test (i.e., BOD_5).

Community — A set of taxa coexisting at a specified spatial or temporal scale.

Concentration Units Table

Concentration Units	Abbreviation	Units
Parts per million	ppm	mg/kg or µg/g or mg/L
Parts per billion	ppb	µg/kg or ng/g or µg/L
Parts per trillion	ppt	ng/kg or pg/g or ng/L
Parts per quadrillion	ppq	pg/kg or fg/g or pg/L

Conductivity — A numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions, their total concentration, mobility, valence and relative concentrations, and on the temperature of measurement.

Dioxins/Furans — Polychlorinated dibenzo-para-dioxins (PCDDs) and dibenzofurans (PCDFs) are often simply called dioxins, although they are two separate groups of substances with similar effects. There are 210 different compounds, of which 17 are the most toxic.

DO	Dissolved oxygen, the gaseous oxygen in solution with water. At low concentrations it may become a limiting factor for the maintenance of aquatic life. It is normally measured in milligrams/litre, and is widely used as a criterion of receiving water quality. The level of dissolved oxygen which can exist in water before the saturation point is reached is primarily controlled by temperature, with lower temperatures allowing for more oxygen to exist in solution. Photosynthetic activity may cause the dissolved oxygen to exist at a level which is higher than this saturation point, whereas respiration may cause it to exist at a level which is lower than this saturation point. At high saturation, fish may contract gas bubble disease, which produces lesions in blood vessels and other tissues and subsequent physiological dysfunctions.
EC*p*	A point estimate of the concentration of test material that causes a specified percentage effective toxicity (sublethal or lethal). In most instances, the EC*p* is statistically derived by analysis of an observed biological response (e.g., incidence of nonviable embryos or reduced hatching success) for various test concentrations after a fixed period of exposure. EC25 is used for the rainbow trout sublethal toxicity test.
Gonad	A male or female organ producing reproductive cells or gametes (i.e., female ovum, male sperm). The male gonad is the testis, the female gonad is the ovary.
IC*p*	A point estimate of the concentration of test material that causes a specified percentage impairment in a quantitative biological test which measures a change in rate, such as reproduction, growth, or respiration.
LC_{50}	Median lethal concentration. The concentration of a substance that is estimated to kill half of a group of organisms. The duration of exposure must be specified (e.g., 96-hour LC_{50}).
Macroinvertebrates	Those invertebrate (without backbone) animals that are visible to the eye and retained by a sieve with 500 µm mesh openings for freshwater, or 1,000 µm mesh openings for marine surveys (EEM methods).
Negative Control	Material (e.g., water) that is essentially free of contaminants and of any other characteristics that could adversely affect the test organism. It is used to assess the "background response" of the test organism to determine the acceptability of the test using predefined criteria.

Organochlorine	Chlorine that is attached to an organic molecule. The amount present is expressed as the weight of the chlorine. There are thousands of such substances, including some that are manufactured specifically as pesticides because of their toxicity.
pH	A measure of the acid or alkaline nature of water or some other medium. Specifically, pH is the negative logarithm of the hydronium ion (H_30^+) concentration (or more precisely, activity). Practically, pH 7 represents a neutral condition in which the acid hydrogen ions balance the alkaline hydroxide ions. The pH of the water can have an important influence on the toxicity and mobility of chemicals in pulpmill effluents.
Plume	The main pathway for dispersal of effluent within the receiving waters, prior to its complete mixing.
Population	A group of organisms belonging to a particular species or taxon, found within a particular region, territory or sampling unit. A collection of organisms that interbreed and share a bounded segment of space.
ppt	Parts per thousand.
Quality Assurance (QA)	Refers to the externally imposed technical and management practices which ensure the generation of quality and defensible data commensurate with the intended use of the data; a set of operating principles that, if strictly followed, will produce data of known defensible quality.
Quality Control (QC)	Specific aspect of quality assurance which refers to the internal techniques used to measure and assess data quality and the remedial actions to be taken when data quality objectives are not realized.
Reference Toxicant	A chemical of quantified toxicity to test organisms, used to gauge the fitness, health, and sensitivity of a batch of test organisms.
Salinity	A measure of the quantity of dissolved salts in seawater - in parts per thousand (ppt) by weight.
SD	Standard deviation.
SE	Standard error.

Secondary Treatment	A stage of purification of a liquid waste in which micro-organisms decompose organic substances in the waste. In the process, the micro-organisms use oxygen. Oxygen usually is supplied by mechanical aeration and/or large surface area of treatment ponds (lagoons). Most secondary treatment also reduces toxicity.
Sentinel Species	A monitoring species selected to be representative of the local receiving environment.
Stressor	An environmental factor or group of factors eliciting a response by a community.
Sublethal	A concentration or level that would not cause death. An effect that is not directly lethal.
T_4CDD	2,3,7,8-tetrachlorodibenzo-para-dioxin, the most toxic dioxin.
TEQ	Toxic Equivalency (see Total-TEQ).
TN	Total nitrogen.
TOC	Total organic carbon (TOC).
Total-TEQ	TEQs are calculated by multiplying the concentration of each congener with its respective International Toxicity Equivalency Factor (ITEF), to normalize concentrations to the level that would be produced by an equivalent amount of 2,3,7,8-T_4CDD, then summing all the concentrations.
TSS	Total suspended solids (TSS) is a measurement of the oven dry weight of particles of matter suspended in the water which can be filtered through a standard filter paper with pore size of 0.45 micrometres.
Turbidity	Turbidity in water is caused by the presence of matter such as clay, silt, organic matter, plankton, and other microscopic organisms that are held in suspension.
v/v	volume/volume - used to define dilution ratios for two liquids.

APPENDICES

Appendix A1

Sublethal Toxicity Data and Calculations

Table A1.1 Howe Sound Pulp and Paper, Sublethal Effluent Toxicity Test Results, Cycle Five.

Testing Period	Project Number	Effluent Description (final, cooling, etc.)	Collection Date yyyymmdd	Consultant/ Laboratory	Species Tested	Test type S=Survival, G=Growth, R=Reproduction	Flag LC50% > for greater than 100%	LC50 %	LC50 Lower 95% cl	LC50 Upper 95% cl	Flag EC25 or IC25% > for greater than 100%	EC25 or IC25 %	EC25 or IC25 Lower 95% cl	EC25 or IC25 Upper 95% cl	Comments
Winter 2006	pp1067	final	20060403	Cantest	Strongylocentrotus purpuratus	R						22.6	19.8	26.2	Cycle Four. Excluded from Cycle Four report because results were not available before report finalized
Winter 2007	pp1067	final	20070430	Cantest	Atherinops affinis	S	>	100							One replicate was accidentally seeded with 6 fish; the statistics for this replicate were modified to be based upon 6 fish.
Winter 2007	pp1067	final	20070430	Cantest	Atherinops affinis	G					>	100			
Winter 2007	pp1067	final	20070710	Cantest	Dendraster excentricus	R						80.1			re-test
Winter 2007	pp1067	final	20070430	Saskatchewan Research Council	Champia parvula	R						5.77	2.76	10.54	
Summer 2007	pp1067	final	20070904	Cantest	Atherinops affinis	S	>	100							
Summer 2007	pp1067	final	20070904	Cantest	Atherinops affinis	G					>	100			
Summer 2007	pp1067	final	20070904	Cantest	Dendraster excentricus	R						39.9	34.56	70.78	
Summer 2007	pp1067	final	20070904	Saskatchewan Research Council	Champia parvula	R						4.31	1.53	7.27	
Winter 2008	pp1067	final	20080325	Cantest	Atherinops affinis	S	>	100							
Winter 2008	pp1067	final	20080325	Cantest	Atherinops affinis	G					>	100			
Winter 2008	pp1067	final	20080331	Cantest	Strongylocentrotus purpuratus	R						30.62	29.61	31.52	
Winter 2008	pp1067	final	20080325	Saskatchewan Research Council	Champia parvula	R						4.52	1.06	11.61	
Summer 2008	pp1067	final	20081208	Cantest	Strongylocentrotus purpuratus	R						25.4	17.4	33.9	
Summer 2008	pp1067	final	20090309	AquaTox Testing & Consulting Inc.	Champia parvula	R						1.31	0.77	1.57	Retest - poor reproduction in culture
Winter 2009	pp1067	final	20090407	Cantest	Strongylocentrotus purpuratus	R						16.6	15.8	18	
Winter 2009	pp1067	final	20090714	AquaTox Testing & Consulting Inc.	Champia parvula	R						1.8	1	2.5	Retest - poor reproduction in culture
Summer 2009	pp1067	final	20091117	Cantest	Strongylocentrotus purpuratus	R						30.3	25.6	30.4	
Summer 2009	pp1067	final	20091117	Saskatchewan Research Council	Champia parvula	R						1.44	0	2.49	

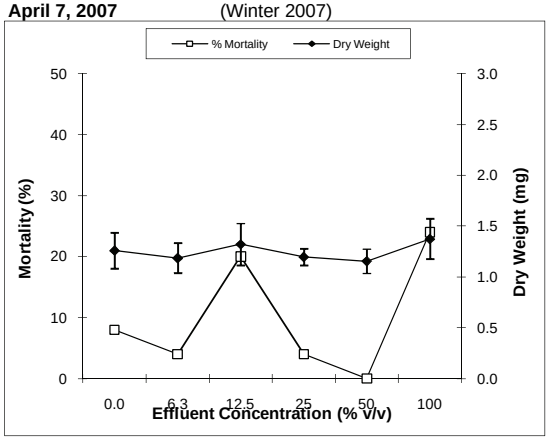
= Cycle Four results

Table A1.2 **Howe Sound Pulp and Paper Limited Partnership - Calculation of geomeans and potential zones of sublethal effect.**

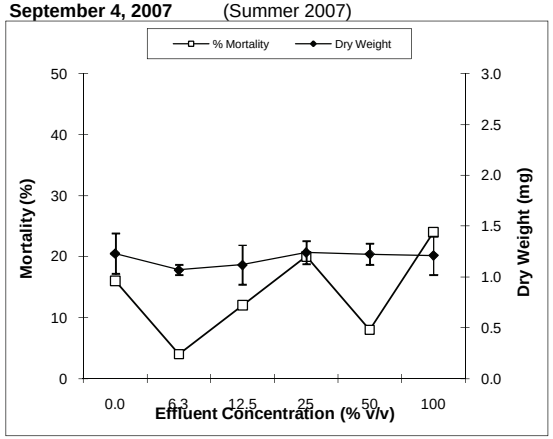
	Fish										Invertebrate					Algae				
	Survival					*Growth*					*Fertilization*					*Reproduction*				
	Cycle 1 LC50	Cycle 2 LC50	Cycle 3 LC50	Cycle 4 LC50	Cycle 5 LC50	Cycle 1 IC25	Cycle 2 IC25	Cycle 3 IC25	Cycle 4 IC25	Cycle 5 IC25	Cycle 1 IC25	Cycle 2 IC25	Cycle 3 IC25	Cycle 4 IC25	Cycle 5 IC25	Cycle 1 IC25	Cycle 2 IC25	Cycle 3 IC25	Cycle 4 IC25	Cycle 5 IC25
	70.9	67	67	100	100	70.9	67	67	100	100		23.01	5.74	10.94	80.1	17.6	28.21	7.49	11.17	5.77
	69	67	67	100	100	69	67	67	100	100		17.92	9.89	37.65	39.9	6.4	18.4	11.29	5.29	4.31
	65.8	67	67	100	100	65.8	67	67	100	100	13.3	11.39	22.02	3.87	30.62	13.2	32.76	25.56	6.57	4.52
	65.4	67	67	100		65.4	67	67	100		9.3	16.82	49.7	67	25.4	63.4	7.12	22.65	10.45	1.31
		68	67	100			68	67	100			13.17	35.37	22.6	16.6		20.8	28.49	16.84	1.8
			100	100				100	100				62.37	15.8	30.3			19.35	16.47	1.44
			100					100					17.96					38.23		
			100					100					62.22					17.84		
Geomean	67.74	67.20	77.86	100.00	100.00	67.74	67.20	77.86	100.00	100.00	11.12	15.97	25.01	18.35	32.82	17.5	19.1	19.2	10.2	2.7
SE	1.3	0.2	6.0	0.0	0.0	1.3	0.2	6.0	0.0	0.0	2.0	2.0	8.0	9.4	9.1	13.0	4.4	3.4	2.0	0.8
1% effluent zone (m)	2000																			
Zone of potential effect (m)	29.5	29.8	25.7	20.0	20.0	29.5	29.8	25.7	20.0	20.0	179.8	125.2	80.0	109.0	60.9	114.1	104.9	104.2	196.1	742.6

Figure A1.1 Mean (± SD) percent mortality and dry weight of topsmelt *(Atherinops affinis)* exposed to final effluent and control water, Howe Sound Pulp and Paper, EEM Cycle Five.

April 7, 2007 (Winter 2007)



September 4, 2007 (Summer 2007)



March 25, 2008 (Winter 2008)

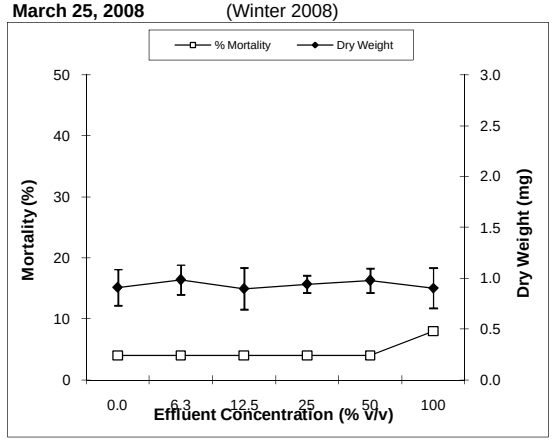
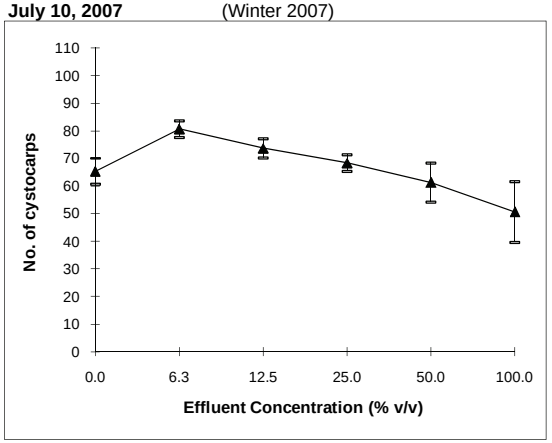


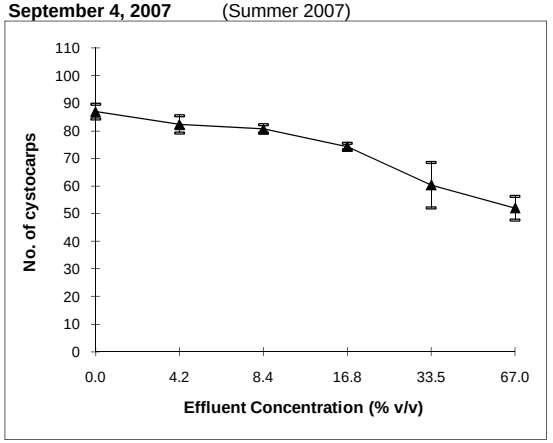
Figure A1.2 Mean (± SD) number of echinoderm eggs fertilized when exposed to final effluent and control water, Howe Sound Pulp and Paper, EEM Cycle Five.

July 10, 2007 (Winter 2007)



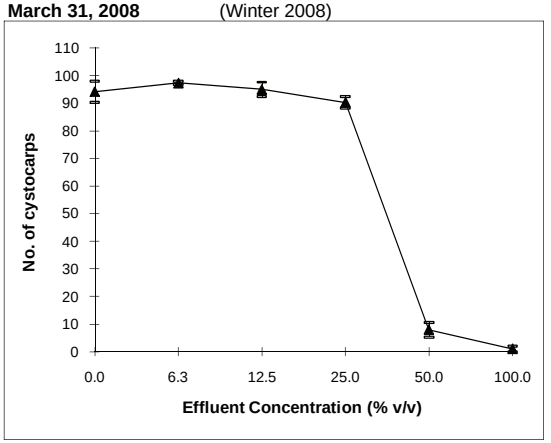
Dendraster excentricus

September 4, 2007 (Summer 2007)



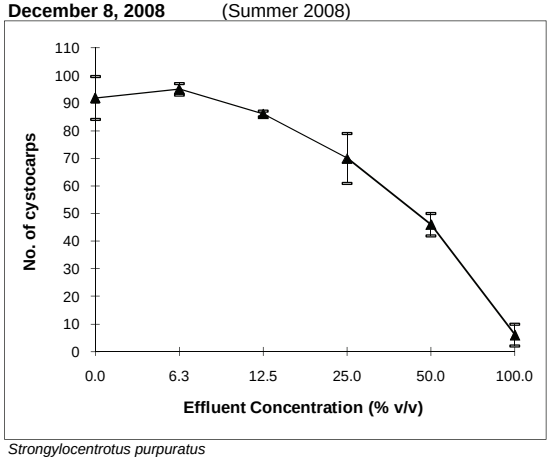
Dendraster excentrotus

March 31, 2008 (Winter 2008)



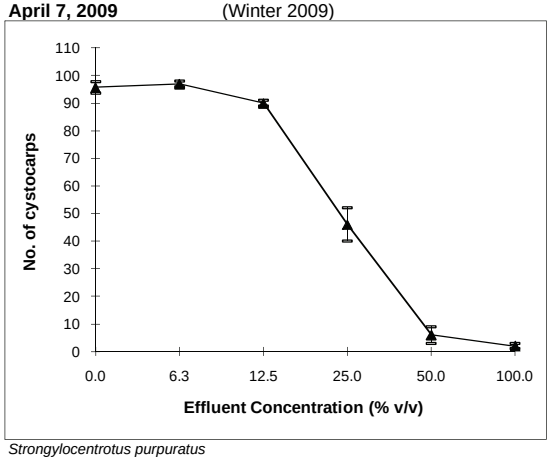
Strongylocentrotus purpuratus

December 8, 2008 (Summer 2008)



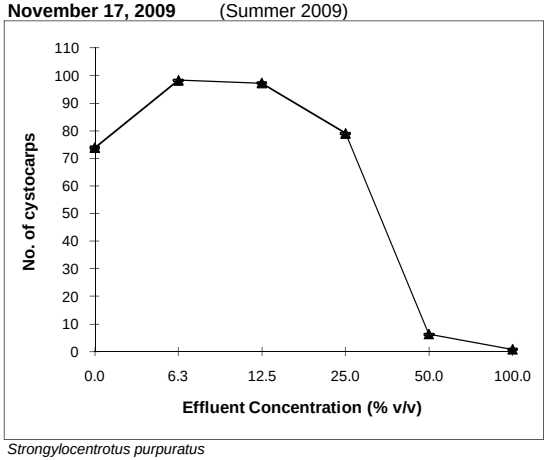
Strongylocentrotus purpuratus

April 7, 2009 (Winter 2009)



Strongylocentrotus purpuratus

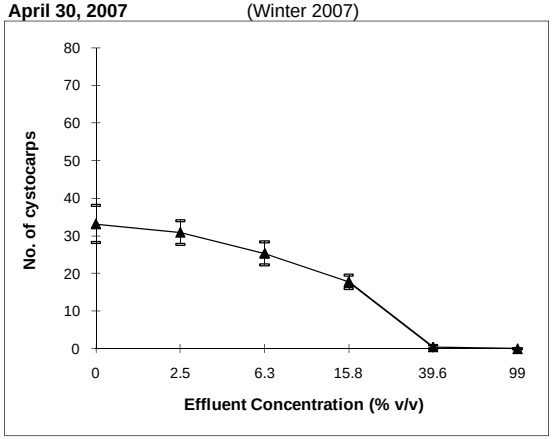
November 17, 2009 (Summer 2009)



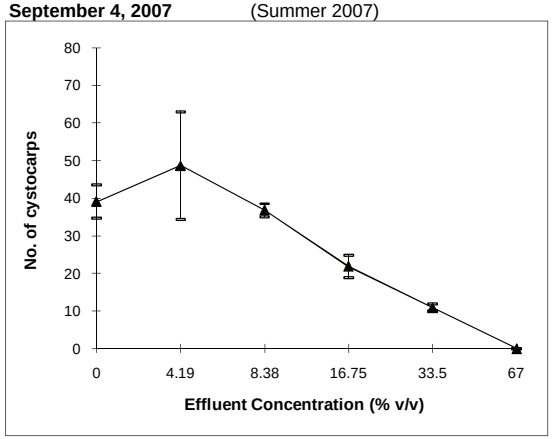
Strongylocentrotus purpuratus

Figure A1.3 **Mean (± SD) number of cystocarps produced by an alga (*Champia parvula*) exposed to final effluent and control water, Howe Sound Pulp and Paper, EEM Cycle Five.**

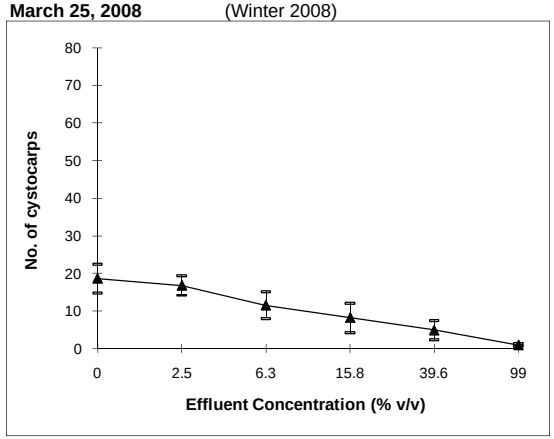
April 30, 2007 (Winter 2007)



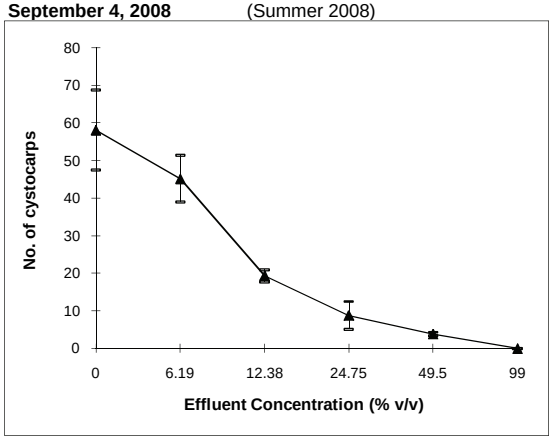
September 4, 2007 (Summer 2007)



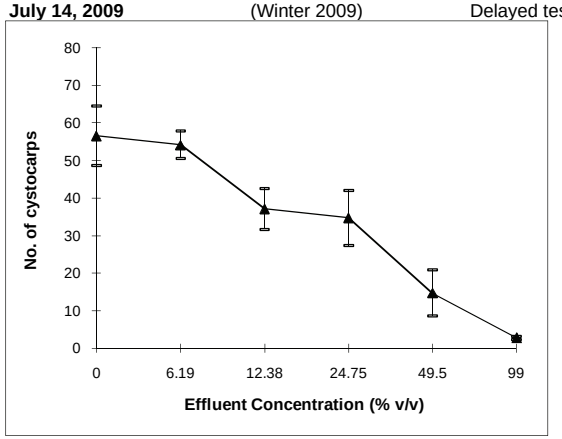
March 25, 2008 (Winter 2008)



September 4, 2008 (Summer 2008)



July 14, 2009 (Winter 2009) Delayed test



November 17, 2009 (Summer 2009)

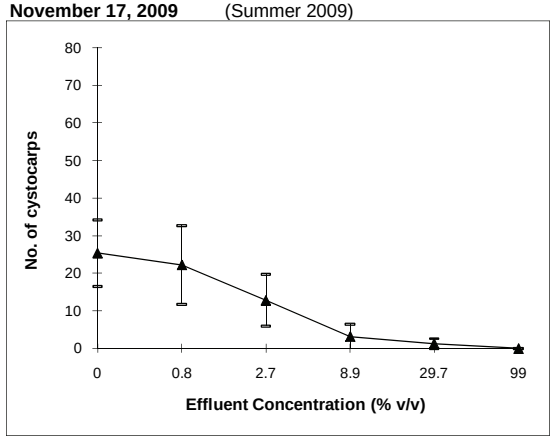
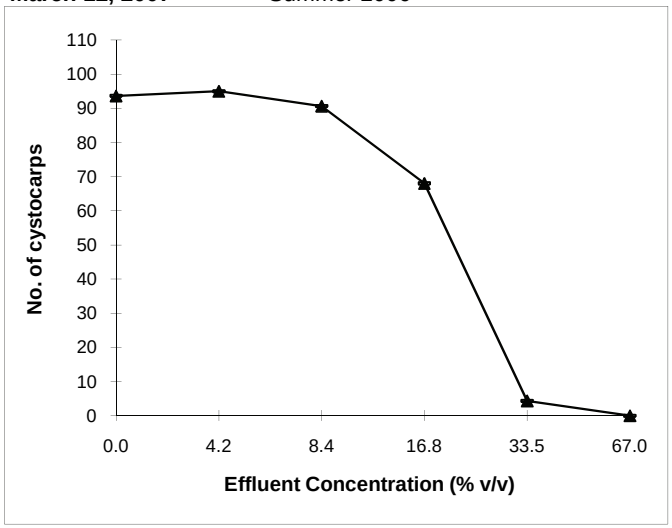


Figure A1.4 Mean (± SD) number of echinoderm eggs fertilized when exposed to final effluent and control water, Howe Sound Pulp and Paper, EEM Cycle Four.

March 12, 2007 Summer 2006



Strongylocentrotus purpuratus

Appendix A2

Cantest: Extended Topsmelt Sublethal Toxicity Test Report

28 DAY EARLY LIFE STAGE TEST USING TOPSMELT

ON FINAL EFFLUENT FROM HOWE SOUND PULP & PAPER

Prepared for:
Hatfield Consultants Ltd.
#200 - 850 Harbourside Drive
North Vancouver, BC,
V7P 0A3

Prepared by:
Ecotoxicology Group
Cantest Ltd.
(Cantest is a Maxxam Analytics Company)

Project No. 2-11-691

August 2009

4606 Canada Way, Burnaby, BC, V5G 1K5
Tel: 604 734 7276 Fax: 604 731 2386

SUMMARY

This early life stage test was initiated with fertilised Topsmelt (*Atherinops affini*s) eggs which were exposed to 5 concentrations, 100, 50, 25, 12.5, and 6.25 % of pulp mill effluent during embryo development, hatching and larval development. The eggs hatched between day 7 and 11 of the test and feeding of *Artemia* (brine shrimp) commenced.

Due to high mortality in the controls this static renewal test was terminated after 13 days. Statistics were not performed on these results since the data is questionable due to high control mortality.

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SECTION

1 INTRODUCTION

Hatfield Consultants Ltd. requested Cantest Ltd. to perform an extended sublethal early life stage test using a marine fish species for Cycle Five of the Environment Effects Monitoring Program. There is not standard test method recognized by Environment Canada for this type of test. In Cycle Three our lab conducted several modified tests on pulp mill effluent using a modified USEPA method for a 7 day survival and growth Topsmelt test. This test proved problematic in the past but Cantest and Hatfield were willing to try it again with some modifications in the test procedure to overcome past problems. Unfortunately excessive mortality in most of the concentrations including the controls made completion of the 28 day test unattainable. Despite best efforts, the test was terminated on day 13 when the control mortality exceeded the maximum allowed. The results of this test and the accompanying reference toxicant test are presented in this report.

SECTION

2 EFFLUENT INFORMATION

2.1 Collection, Shipping and Holding of Effluent

The test was initiated on August 14 and terminated on August 27, 2009. Effluent samples for the test were collected and shipped at the beginning of each week. The "Toxicity Test Request Sheets" with the date and time of collection are found in the Appendix. The first set of samples was collected in 6 – 20 L carboys and the second set of samples was collected in 3 – 20 L carboys. Both sets of samples were shipped to Cantest Ltd. and arrived on the following day. Upon arrival at Cantest Ltd. the temperature of the sample was taken and recorded. The sample was placed in a 4°C room until needed.

The login identification numbers, dates of sample collection and arrival and temperatures upon arrival for the effluent samples are summarised in Table 1-1.

Table 2-1: Final Effluent Sample Information

Login ID	Date of Collection	Dates of Sample Usage	Date of Sample Arrival	Arrival Temp. (°C)
908130064	Aug 12 , 2009	Aug 14 - 20	Aug 13, 2009	21.5
908200059	Aug 19, 2009	Aug 21 – Aug 27	Aug 20 , 2009	24.6

The first use of each sample was within the 3 day holding period.

2.2 Salinity Adjustment of the Effluent

The salinity of the samples ranged from 1 to 1.5 ppt. Artificial sea salts were used to adjust the salinity of sample in accordance with the present EEM recommendations for salinity adjustment. Using sea salts instead of hypersaline brine allows the testing of a higher concentration of effluent, 100% instead of 67%. Oceanpure sea salts were used in this test. This method is based on the "Revised Procedures for Adjusting Salinity of Effluent Samples for Marine Sublethal Toxicity Testing Conducted under Environmental Effects Monitoring (EEM) Programs" (Environment Canada 2001).

The day before (~ 16 hours) the container of sample (cubitainer or "bag") was used, artificial sea salts were added to 10 L of sample to bring the salinity to 30 ± 2‰. Each salinity-adjusted bag

was used for 2 to 3 days. The records of the salinity adjustment are on the “Marine EEM Salinity Adjustment Using Dry Salts Datasheet” found in the Appendix.

The sea salt was added to the effluent in a cubitainer and was shaken vigorously. The pH of the effluent was taken before and after the salt was added, but before ageing. The salinity was measured and adjusted as necessary using more salt or effluent. The salinity-adjusted effluent was placed in a sealed carboy in the cold room in the dark at 4 $\pm$ 2°C. All air was removed from the carboy. On the day of use, an aliquot of effluent required for the test was removed from well-shaken carboy and adjusted to test temperature.

SECTION

3 TEST CONDITIONS

3.1 Test Methods

The test method followed was the “Standard Operating Procedure for the Topsmelt (*Atherinops affinis*) 28 Day Survival and Growth Test”, BC Research SOP 1303, version 2.0. This test method was based on the information provided in the US Environmental Protection Agency methodology document, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms" (USEPA 1995) and the chapter “Methods for Spawning, Culturing, and Conducting Toxicity Tests with Early Life-Stages of Estuarine and Marine Fishes” (Middaugh 1993).

3.2 Acclimation and Holding Conditions of Test Organisms

Fertilised eggs, less than 24 hours old, were shipped to Cantest Ltd. from Aquatic Bio-Systems, Fort Collins, CO. The eggs were acclimated at Cantest Ltd. to the test conditions for temperature, photo-period, and water, for a day before starting the test.

3.3 Test Facilities

3.3.1 Temperature

The test was conducted in a temperature controlled room (20 $\pm$ 1°C). The laboratory temperature, using a min/max thermometer, was recorded daily on the “Daily Records of Storage Temperature” data sheet.

3.3.2 Lighting and Photo-period

Light intensity was provided by broad spectrum fluorescent lights which had an intensity of 54-95 foot candles which was within the required levels of 50-100 foot candles. The photoperiod was a 16:8 hour light:dark cycle.

3.4 Preparation of Test Solutions

Approximately 1 hour before test initiation and the daily renewal of the test solutions 2 L of sample was warmed to 20 ± 1°C. The sample was agitated thoroughly just before using.

Sample pH, dissolved oxygen, temperature, and salinity were measured before the preparation of sample solutions on each day of the test. These initial measurements were recorded on the "*Atherinops affinis* Larval Survival and Growth Test – Daily Test Conditions" datasheet which is in the Appendix.

There were five test concentrations using the artificial seawater (30 ± 2‰) for dilution. The test concentrations were prepared in 1 L volumes (Table 2-1).

Table 3-2: Volumes of Seawater & Salinity Adjusted Effluent used to Prepare Test Solutions

Concentration (%v/v)	Volume of Salinity Adjusted Sample (ml)	Volume of Seawater H_20 (ml)
100	1000	0
50	400	600
25	160	840
12.5	64	936
6.25	25	975
Total	1649	3351

The test was conducted in 250-ml borosilicate glass beakers with 5 replicates per concentration and 5 fish per replicate. The volume of solution in each test vessel was 200ml and the depth of the solution was 6.5 cm. The eggs were placed in stainless steel mesh baskets suspended in the test vessels. The eggs were placed in baskets to prevent accidental siphoning out of eggs during water change. The test vessels were randomised and covered with a Plexiglas sheet to exclude the possibility of contamination and evaporation.

3.5 Preparation of Controls & Dilution Water

The salinity of the salt control/dilution water was within ± 2 ‰ of the test salinity. The salt control/dilution water was prepared by adding artificial sea-salt to deionized water. The salinity was measured and adjusted as necessary using more salt or deionized water. The newly prepared water was aerated vigorously for a minimum of 24 hours before using in the test. This water was stored for up to 7 days at (4 ± 2°C) following preparation. The day prior to use an aliquot of control water was aerating in the test room at test temperature. The records of the mixing procedure are found in the Appendix as "Salt and Salt Water Control Salinity Adjustment Using Dry Salts Datasheet".

3.6 Test Initiation

The Topsmelt eggs were attached to the substrate (yarn) on which they were shipped. A cluster of yarn with the attached eggs was transferred to a large Petri dish with seawater. The Petri dish was placed under a dissecting microscope. The eggs were attached to each other in large clusters similar to grapes. Small scissors were used to cut the yarn and chorionic fibrils that attached the eggs to each other and the yarn. Whenever possible 5 eggs were left connected by the fibrils. Occasionally separated eggs were used to achieve the total of 5 eggs per replicate. This procedure was done while keeping the eggs immersed in water. Five Topsmelt eggs were added to each test vessel. The Topsmelt eggs were counted again as they were added to the vessels. If any eggs appeared dead (i.e. opaque) they were replaced at this time.

3.7 Test Maintenance

3.7.1 Daily Cleaning of Test Vessels

Each day the uneaten and dead *Artemia* and other debris, including dead Topsmelt, were removed from the test vessels with a siphon hose, fitted with a 10 ml glass pipette. The water level was lowered to around 50 ml, removing approximately 80% of the test solution. Any Topsmelt that were accidentally removed during cleaning were returned to the test vessel and noted on the “Test Observations” data sheet.

3.7.2 Test Solution Renewal

The test concentrations were renewed daily using freshly prepared solutions, immediately after cleaning each test vessel. New test solution was added slowly, refilling each vessel to 200 ml, by carefully pouring the solution down the side of the beaker. This minimised the disturbance to the Topsmelt.

If the Topsmelt were difficult to see due to dark cloudy effluent, a light table was utilised as needed.

3.7.3 Feeding

Feeding began as soon as the eggs began to hatch, on the 7th day of the test. Test organisms were fed newly hatched brine shrimp, *Artemia* nauplii, (<24 hours old) twice daily on the remaining days of the test. The larvae were feed two hours before, (the solution change) and once again after all solutions were changed. The feeding records are on the “Test Observations“ data sheets, and the *Artemia* preparation records are found on the “*Artemia* Counts” data sheets, both of which are in the Appendix.

3.8 Measurements and Observations during the Test

3.8.1 Chemical and Physical Determinations

Salinity, pH, temperature, and dissolved oxygen were measured at the initiation and termination of each 24 h exposure period in all concentrations and the control and were recorded on the "Daily Test Conditions" data sheet. The initial measurements were taken after the solutions were mixed and before the daily solution renewal in 1L reagent bottles. The final measurements were taken in separate beakers from the test vessels after the water change, to avoid unnecessary disturbances and damage to the Topsmelt. The measurements were taken in a different set of replicates each day (i.e. Day 1-A replicate, Day 2-B replicate, etc.). The replicate used was recorded on the "Test Observations" data sheet. These data sheets are in the Appendix.

Dissolved oxygen was also measured in the sample at the beginning of each day before the test solutions were mixed. If the DO in the sample was below 40% saturation, it was pre-aerated until 40% saturation was obtained and recorded on the "Test Observations" data sheet.

3.8.2 Biological Observations

The numbers of live Topsmelt in each test vessel were recorded daily on the "Survival Data" sheet, and any dead Topsmelt were discarded. If the eggs turned opaque (white or pink) they were considered dead and were removed. Topsmelt larvae were considered dead when they did not show any swimming motion after they were stimulated by a gentle jet of test solution from a 4-mm glass pipette. Deformity, loss of equilibrium or abnormal swimming behaviour were also recorded.

The numbers of hatched Topsmelt eggs in each test vessel were recorded daily on the "Survival Data" sheet.

3.9 Test Endpoints and Calculation

Endpoints could not be calculated since the controls exceeded the maximum allowed mortality on day 13.

Before the test ended an effect was seen in the 100% concentration. A delay in hatching and a lower percentage of hatched larvae was noted in the 100% concentration when compared to the controls, 8.7 days (pooled controls) versus 10 days and 70% hatched in controls versus 44% hatched in the highest concentration of effluent.

The hatching and survival data is presented in several tables and graphs within this report.

SECTION

4 QUALITY ASSURANCE/QUALITY CONTROL

The Topsmelt eggs were shipped to our lab in less than 24 hours. The mortality of the batch of eggs did not exceed 10% of the entire shipment.

The BC Research SOP (Standard Operating Procedure) set the test validity criteria at ≥65% control survival. This test criterion was set in agreement with EC based on criteria used for the 30-day rainbow trout embryo/alevin test (EC 1998). The criteria used for the 28 day test is lower than criteria used for the EC (1998) and USEPA (1995) 7-day Topsmelt tests (≥80% survival) because of the extended duration of the exposure. The survival in the controls was 64%.

All instruments were calibrated daily to ensure that the water quality measurements that were taken were accurate. These records are kept on file in the laboratory.

A reference toxicant test using reagent grade sodium dodecyl sulfate (SDS) was conducted on the eggs to assess the sensitivity of the Topsmelt and the precision of the data produced. The geometric mean for the previous LC50 values was 7.6 mg/L and the two standard deviations of that mean were 5.1 and 10.1 mg/L. The estimated LC50 for the reference toxicant test conducted on this batch of eggs was 6.5 mg/L with 95% confidence limits of 3.4 and 6.8. Therefore the LC50 of this test fell within 2 standard deviation of the mean of previous tests and is considered valid.

SECTION

5 RESULTS

5.1 Water Quality Measurements

The overlying water measurements for the Topsmelt test are summarised in Table 4-3.

Table 5-3: Ranges of Test Water Quality Measurements for Final Effluent

Sample Concentration (%v/v)	Temperature (°C)	DO (mg/L)	pH	Salinity (‰)
Control (0)	19.0 – 20.1	7.2 - 8.1	7.6 – 8.0	30 - 31
6.25	19.0 – 19.9	6.9 – 8.1	7.5 - 8.1	30 - 31
12.5	19.0 – 20.1	7.0 – 8.2	7.3 – 8.1	30 - 31
25	19.0 – 20.6	7.0 – 8.2	7.0 – 8.0	30 - 31
50	19.0 – 20.9	6.1 – 7.9	6.8 – 8.1	30 - 31
100	19.0 – 21.0	6.0 – 8.0	6.7 – 8.1	29 - 31

The water quality values were consistently within the test criteria and acceptable ranges for this test organism.

5.2 Summary of Test Results

Statistics were not performed on these results since the data is questionable due to high control mortality. A summary of egg and larvae survival and mean hatching time is included in Table 4-4.

Table 5-4: Mean and Standard Deviations of Egg and Larvae Survival and Larvae Growth and Time to Complete Hatching

Concentration	Egg Survival		Larvae Survival		Larvae Growth (mg)		Time to Complete Hatching (days)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sea Water Control (0%)	72%	23%	72%	23%	No Data		8.4	0.5
Salt Water Control (0%)	68%	11%	56%	22%			9.0	1.2
6.25 %	92%	11%	76%	17%			9.2	0.8
12.5 %	56%	22%	40%	14%			8.8	1.3
25%	60%	40%	44%	46%			8.8	0.5
50%	68%	18%	52%	30%			9.0	0.7
100%	44%	30%	40%	32%			10.5	0.6

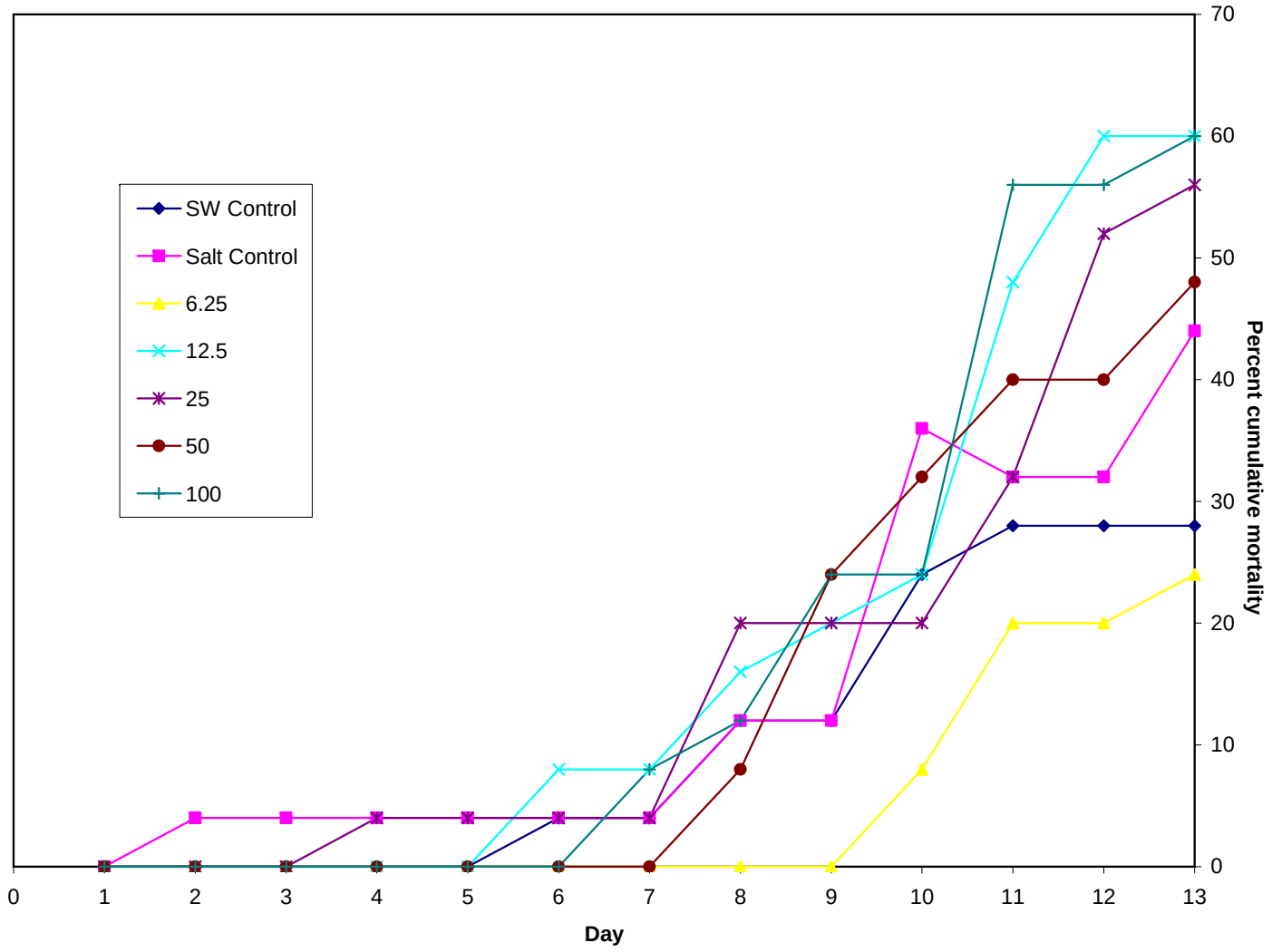

Figure 1-5: Percent Cumulative Mortality Chart

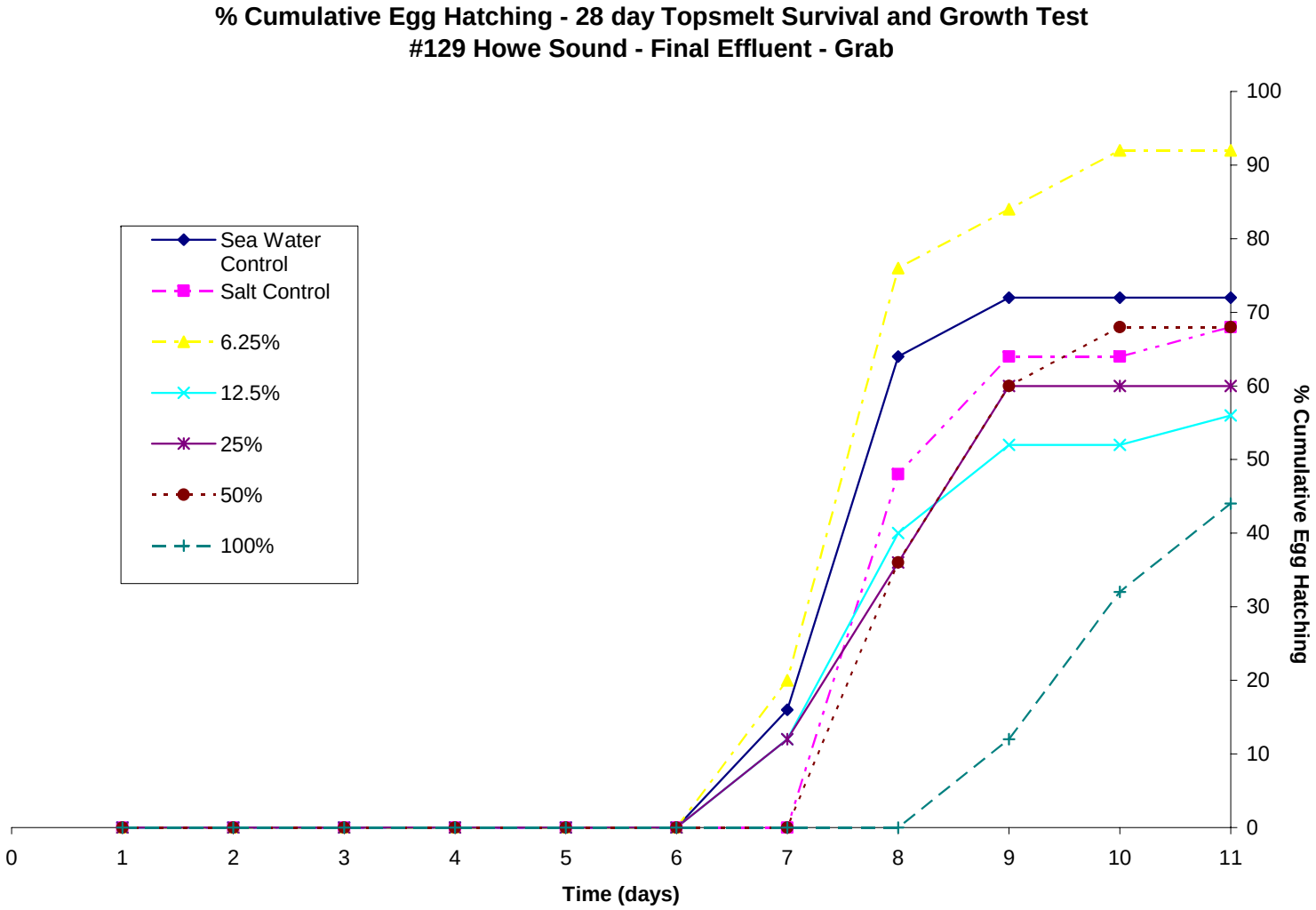

Figure 2-5: Percent Cumulative Egg Hatching

SECTION

6 CONCLUSIONS

The test was terminated after 13 days due to high mortality rate in Salt Controls. The combined mortality rate of the controls was 36.0 % while the maximum allowed in the protocol is 35% mortality. There was also high mortality in most concentrations of the test without a defined dose response indicating a problem with the test organisms or the test protocol. Statistics were not performed on these results since the data is questionable due to high control mortality.

It is unknown what caused the high mortality in this test. It is known that maintaining Topsmelt in these test conditions is difficult and thus has not been routinely performed by ecotoxicology labs.

SECTION

7 LITERATURE CITED

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APPENDIX

Howe Sound - 28 day test - Hatching Data
Aug. 2009

		# Hatched													
Conc. (%v/v)	Replicate #	Hatch 1	Hatch 2	Hatch 3	Hatch 4	Hatch 5	Hatch 6	Hatch 7	Hatch 8	Hatch 9	Hatch 10	Hatch 11	% Hatched	% Mean Hatched	SD (%)
	Date	08/15	08/16	08/17	08/18	08/19	08/20	08/21	08/22	08/23	08/24	08/25			
Sea Water Control	A	0	0	0	0	0	0	1	4	4	4	4	80%	72.0%	22.8%
	B	0	0	0	0	0	0	1	2	2	2	2	40%		
	C	0	0	0	0	0	0	0	2	3	3	3	60%		
	D	0	0	0	0	0	0	2	4	4	4	4	80%		
	E	0	0	0	0	0	0	0	4	5	5	5	100%		
Lab Control	A	0	0	0	0	0	0	0	3	4	4	4	80%	68.0%	11.0%
	B	0	0	0	0	0	0	0	2	2	2	3	60%		
	C	0	0	0	0	0	0	0	0	3	3	3	60%		
	D	0	0	0	0	0	0	0	4	4	4	4	80%		
	E	0	0	0	0	0	0	0	3	3	3	3	60%		
6.25	A	0	0	0	0	0	0	0	3	3	4	4	80%	92.0%	11.0%
	B	0	0	0	0	0	0	1	3	3	4	4	80%		
	C	0	0	0	0	0	0	0	4	5	5	5	100%		
	D	0	0	0	0	0	0	2	5	5	5	5	100%		
	E	0	0	0	0	0	0	2	4	5	5	5	100%		
12.5	A	0	0	0	0	0	0	2	3	3	3	3	60%	56.0%	21.9%
	B	0	0	0	0	0	0	1	4	4	4	4	80%		
	C	0	0	0	0	0	0	0	1	2	2	3	60%		
	D	0	0	0	0	0	0	0	1	1	1	1	20%		
	E	0	0	0	0	0	0	0	1	3	3	3	60%		
25	A	0	0	0	0	0	0	2	3	4	4	4	80%	60.0%	40.0%
	B	0	0	0	0	0	0	0	0	0	0	0	0%		
	C	0	0	0	0	0	0	0	3	4	4	4	80%		
	D	0	0	0	0	0	0	1	2	2	2	2	40%		
	E	0	0	0	0	0	0	0	1	5	5	5	100%		
50	A	0	0	0	0	0	0	0	1	3	3	3	60%	68.0%	17.9%
	B	0	0	0	0	0	0	0	0	0	2	2	40%		
	C	0	0	0	0	0	0	0	1	4	4	4	80%		
	D	0	0	0	0	0	0	0	3	4	4	4	80%		
	E	0	0	0	0	0	0	0	4	4	4	4	80%		
100	A	0	0	0	0	0	0	0	0	0	2	2	40%	44.0%	29.7%
	B	0	0	0	0	0	0	0	0	1	2	2	40%		
	C	0	0	0	0	0	0	0	0	0	0	0	0%		
	D	0	0	0	0	0	0	0	0	2	2	3	60%		
	E	0	0	0	0	0	0	0	0	0	2	4	80%		
Analyst		JR	JR	NS	KT	NS	NS	NS	JR	JR	MBB	MG			

	Cumulative % Hatching										
Conc. (%v/v)	1	2	3	4	5	6	7	8	9	10	11
Sea Water Control	0	0	0	0	0	0	16	64	72	72	72
Salt Control	0	0	0	0	0	0	0	48	64	64	68
6.25	0	0	0	0	0	0	20	76	84	92	92
12.5	0	0	0	0	0	0	12	40	52	52	56
25	0	0	0	0	0	0	12	36	60	60	60
50	0	0	0	0	0	0	0	36	60	68	68
100	0	0	0	0	0	0	0	0	12	32	44

Time to complete hatch						Mean	SD
Sea Water Control	8	8	9	8	9	8.4	0.5
Salt Control	9	11	9	8	8	9	1.2
6.25	10	10	9	8	9	9.2	0.8
12.5	8	8	11	8	9	8.8	1.3
25	9		9	8	9	8.75	0.5
50	9	10	9	9	8	9	0.7
100	10	10		11	11	10.5	0.6

Proofed: LS 2010Jan29

Cantest Ltd.

Howe Sound Pulp Paper
Topsmelt 13-day Survival and Growth Test

Survival Data

# Surviving Organisms																	
Conc.	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	%	% Mean	SD
(%v/v)	Date	8/15	8/16	8/17	8/18	8/19	8/20	8/21	8/22	8/23	8/24	8/25	8/26	8/27	Survival	Survival	(%)
Sea Water Control	A	5	5	5	5	5	5	5	5	5	5	4	4	4	80%	72%	23%
	B	5	5	5	5	5	4	4	3	3	2	2	2	2	40%		
	C	5	5	5	5	5	5	5	4	4	3	3	3	3	60%		
	D	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
	E	5	5	5	5	5	5	5	5	5	5	5	5	5	100%		
Salt Control	A	5	5	5	5	5	5	5	5	5	4	4	4	1	20%	56%	22%
	B	5	5	5	5	5	5	5	5	5	2	3	3	3	60%		
	C	5	4	4	4	4	4	4	4	4	3	3	3	3	60%		
	D	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
	E	5	5	5	5	5	5	5	3	3	3	3	3	3	60%		
6.25	A	5	5	5	5	5	5	5	5	5	4	3	3	3	60%	76%	17%
	B	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
	C	5	5	5	5	5	5	5	5	5	5	3	3	3	60%		
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	100%		
	E	5	5	5	5	5	5	5	5	5	5	5	5	4	80%		
12.5	A	5	5	5	5	5	5	5	4	3	3	2	2	2	40%	40%	14%
	B	5	5	5	5	5	5	5	4	4	4	4	2	2	40%		
	C	5	5	5	5	5	3	3	3	3	3	3	2	2	40%		
	D	5	5	5	5	5	5	5	5	5	5	1	1	1	20%		
	E	5	5	5	5	5	5	5	5	5	4	3	3	3	60%		
25	A	5	5	5	5	5	5	5	4	4	4	3	1	0	0%	44%	46%
	B	5	5	5	5	5	5	5	3	3	3	3	0	0	0%		
	C	5	5	5	5	5	5	5	5	5	5	4	4	4	80%		
	D	5	5	5	4	4	4	4	3	3	3	2	2	2	40%		
	E	5	5	5	5	5	5	5	5	5	5	5	5	5	100%		
50	A	5	5	5	5	5	5	5	5	4	3	3	3	3	60%	52%	30%
	B	5	5	5	5	5	5	5	4	2	2	0	0	0	0%		
	C	5	5	5	5	5	5	5	4	4	4	4	4	3	60%		
	D	5	5	5	5	5	5	5	5	4	4	4	4	3	60%		
	E	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
100	A	5	5	5	5	5	5	3	2	2	2	2	2	1	20%	40%	32%
	B	5	5	5	5	5	5	5	5	2	2	2	2	2	40%		
	C	5	5	5	5	5	5	5	5	5	5	0	0	0	0%		
	D	5	5	5	5	5	5	5	5	5	5	3	3	3	60%		
	E	5	5	5	5	5	5	5	5	5	5	4	4	4	80%		
Analyst		JR	JR	NS	KT	NS	NS	NS	JR	JR	MBB	MG	NS	NS			

Cumulative Mortality													
Conc.	Day												
(%v/v)	1	2	3	4	5	6	7	8	9	10	11	12	13
SW Control	0	0	0	0	0	1	1	3	3	6	7	7	7
Salt Control	0	1	1	1	1	1	1	3	3	9	8	8	11
6.25	0	0	0	0	0	0	0	0	0	2	5	5	6
12.5	0	0	0	0	0	2	2	4	5	6	12	15	15
25	0	0	0	1	1	1	1	5	5	5	8	13	14
50	0	0	0	0	0	0	0	2	6	8	10	10	12
100	0	0	0	0	0	0	2	3	6	6	14	14	15

Percent Cumulative Mortality													
Conc.	Day												
(%v/v)	1	2	3	4	5	6	7	8	9	10	11	12	13
SW Control	0	0	0	0	0	4	4	12	12	24	28	28	28
Salt Control	0	4	4	4	4	4	4	12	12	36	32	32	44
6.25	0	0	0	0	0	0	0	0	0	8	20	20	24
12.5	0	0	0	0	0	8	8	16	20	24	48	60	60
25	0	0	0	4	4	4	4	20	20	20	32	52	56
50	0	0	0	0	0	0	0	8	24	32	40	40	48
100	0	0	0	0	0	0	8	12	24	24	56	56	60

Proofed: LS 2010Jan29

Mean and Standard Deviations of Egg and Larvae Survival and Time to Complete Hatching

Concentration	Egg Survival		Larvae Survival		Larvae Growth (mg)		Time to Complete Hatching (days)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sea Water Control (0%)	72.0%	22.8%	72%	23%			8.4	0.5
Salt Control (0%)	68.0%	11.0%	56%	22%			9	1.2
6.25%	92.0%	11.0%	76%	17%			9.2	0.8
12.5%	56.0%	21.9%	40%	14%			8.8	1.3
25.0%	60.0%	40.0%	44%	46%			8.75	0.5
50%	68.0%	17.9%	52%	30%			9	0.7
100%	44.0%	29.7%	40%	32%			10.5	0.6

Larvae Survival on day 13				Mean	SD
Sea Water Control	A	4	80%	72%	23%
	B	2	40%		
	C	3	60%		
	D	4	80%		
	E	5	100%		
Salt Control	A	1	20%	56%	22%
	B	3	60%		
	C	3	60%		
	D	4	80%		
	E	3	60%		
6.25	A	3	60%	76%	17%
	B	4	80%		
	C	3	60%		
	D	5	100%		
	E	4	80%		
12.5	A	2	40%	40%	14%
	B	2	40%		
	C	2	40%		
	D	1	20%		
	E	3	60%		
25	A	0	0%	44%	46%
	B	0	0%		
	C	4	80%		
	D	2	40%		
	E	5	100%		
50	A	3	60%	52%	30%
	B	0	0%		
	C	3	60%		
	D	3	60%		
	E	4	80%		
100	A	1	20%	40%	32%
	B	2	40%		
	C	0	0%		
	D	3	60%		
	E	4	80%		
Analyst		MG			

Egg Survival on day 11				Mean	SD
Sea Water Control	A	4	80%	72.0%	22.8%
	B	2	40%		
	C	3	60%		
	D	4	80%		
	E	5	100%		
Salt Control	A	4	80%	68.0%	11.0%
	B	3	60%		
	C	3	60%		
	D	4	80%		
	E	3	60%		
6.25	A	4	80%	92.0%	11.0%
	B	4	80%		
	C	5	100%		
	D	5	100%		
	E	5	100%		
12.5	A	3	60%	56.0%	21.9%
	B	4	80%		
	C	3	60%		
	D	1	20%		
	E	3	60%		
25	A	4	80%	60.0%	40.0%
	B	0	0%		
	C	4	80%		
	D	2	40%		
	E	5	100%		
50	A	3	60%	68.0%	17.9%
	B	2	40%		
	C	4	80%		
	D	4	80%		
	E	4	80%		
100	A	2	40%	44.0%	29.7%
	B	2	40%		
	C	0	0%		
	D	3	60%		
	E	4	80%		
Analyst		NS			

Proofed : LS 2010 Jan 26

Old report: 2-11-910

Cantest Ltd.

Atherinops affinis **28 Day Survival and Growth Test**
Acclimation and Holding Conditions

Eggs Used for Client #'s: Haw001 Date & Time of Arrival: 2009 Aug 13 @ 1130

Organism Lot #: AB090813 Age upon Arrival: <24hrs

Water (L) per Shipping Bag: ~2L Number of Eggs Ordered: 550+55 = 605

Number of Shipping Bags: 1

Arrival Conditions

Bag ID	# Dead	% Dead	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Analyst
~~AB090813~~			29.0	20.9	7.3	OVER	NS

Daily Conditions During Holding/Acclimation

Bag A	Mortalities		Water Quality				
Date	# Dead	% Dead	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Analyst

Bag B	Mortalities		Water Quality				
Date	# Dead	% Dead	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Analyst

Total Daily Mortalities			
Date	# Dead	% Dead	Analyst
Total			

Comments (e.g.egg appearance, acclimation conditions): **Analyst**

2009 Aug 13 - began aerating @ 11:31 (took WQ first) NS
- 500 ml added @ 1215 NS
- added ~ 4L SW to a clean 20L fish tank, added some overlying water from culture bag, gently poured remaining H_2O + gently pulled strings out of bag + into tank. Air line added. Eggs visible (green) LS

2009 Aug 20: noticed some a couple of topsmelt hatched, NS
WE NS 2009 Aug 20 fed ~3ml of diluted artemia (from Pttm)

AB090813

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel: 970/484-5091 Fax:970/484-2514

ORGANISM HISTORY

DATE: 8/12/09

SPECIES: *Atherinops affinis*

AGE: <24 hour

LIFE STAGE: Embryos

HATCH DATE: Spawned 8/11/09; To hatch 8/16-8/17/09

BEGAN FEEDING: N/A

FOOD: N/A

Water Chemistry Record:	Mean	Range
TEMPERATURE:	23 °C	--
SALINITY/CONDUCTIVITY:	32 ppt	--
TOTAL HARDNESS (as $CaCO_3$):	--	--
TOTAL ALKALINITY (as $CaCO_3$):	182 mg/l	--
pH:	7.76	--

Comments:

Facility Supervisor

9.0

Cantest Ltd. *Atherinops affinis* **28-d Survival and Growth Test**
Test Observations

Client # & Name: HOWOOL / HOWESOUND Pulp & paper Start Date and Time: 2009 Aug 14 @ 16:45
Sample ID: Fe ~~908200059~~, WE NS 2009 Aug 20 End Date: 2009 Aug 27
Analyst(s): J. Ross N Ehergiel K Tomaki WE NS 2009 Aug 20

Date	Day	Conc.	Replicate	Comments (e.g., replicate used for measurements, atypical behaviour of fish, etc.)	Analyst
2009 Aug 14	0	All	All	Used carboy #1 (salinity adjusted). Sample dark	NS
2009 Aug 14	0	All	All	amber, difficult to see through. Warmed up to 19°C.	NS
2009 Aug 14	0	All	All	Did initial WQ. Room lighting - ~~500~~ 50 100ft. WE NS 2009 Aug 14	NS
2009 Aug 14	0	All	All	finished seeding tests @ 4:45 pm	JR
2009 Aug 15	1	All	All	Used carboy #1 (salinity adjusted). Sample dark amber.	JR
2009 Aug 15	1	All	A	Water quality	JR
2009 Aug 15	1	All	All	Water Δ; all eggs appear normal @ 13:30	JR
2009 Aug 16	2	100	All	Used carboy #1 DO: 8.0 @ 20.9°C aerated 14:06 - 14:31	JR
2009 Aug 16	2	100	All	Sample dark amber and salinity adjusted	JR
2009 Aug 16	2	All	B	Water quality	JR
2009 Aug 16	2	All	All	Water Δ @ 16:00; 1 foggy egg in salt Ctrl C.	JR
2009 Aug 17	3	All	All	Used Carboy #2 (salinity adjusted). Sample dark amber	NS
2009 Aug 17	3	All	All	DO = 7.7 @ 20.0 (100% sample). No Aeration.	NS
2009 Aug 17	3	All	C	Water quality. WE, NS 2009 Aug 17	NS
2009 Aug 17	3	All	All	Water Δ @ 1506 . No eggs have hatched yet	NS
2009 Aug 18	4	All	All	Used carboy #2 (salinity Adjusted) Sample dark amber	Kt
↓	4	↓	↓	DO = 7.8 @ 19.8 No aeration required	Kt
↓	4	↓	↓	H_2O Δ @ 1600	Kt
↓	4	D	↓	WQ rep D WE, NS 2009 Aug 19	Kt
2009 Aug 19	5	100%	—	used carboy #~~2~~ 3 (salinity adjusted) sample dark amber.	JR
2009 Aug 19	5	100%	—	~~DO: 7.9 @ 20.5°C aerated 10:35 - 10:40~~ WE, NS 2009 Aug 19 ~~New DO: 7.6 @ 20.2°C~~	JR
2009 Aug 19	5	All	All	DO = 7.6 @ 20.6°C, No Aeration.	NS
2009 Aug 19	5	All	All	H_2O Δ @ 1350 . WQ Rep E.	NS
2009 Aug 20	6	All	All	Used carboy #3 (salinity adjusted). Dark amber sample	NS
2009 Aug 20	6	100%	—	DO = 7.9 @ 20.9°C, No aeration.	NS
2009 Aug 20	6	All	All	H_2O Δ @ 11:10 . WQ Rep A.	NS
2009 Aug 21	7	All	All	Used Carboy #4 (salinity adjusted - new sample)	NS
2009 Aug 21	7	100%	—	Dark amber sample. DO = 7.9 @ 20.6. No Aeration	NS
2009 Aug 21	7	All	All	H_2O Δ @ 1355 . WQ Rep B. Concn of 50%	NS
2009 Aug 21	7	50/100	All	& 100% all reps look smothered by fluffy	NS
2009 Aug 21	7	50/100	All	stuff in sample (debris?). Fed @ 1630, 25µl	NS
2009 Aug 21	7	—	—	per topsmelt hatched. (Note: unhatched in morning	NS
2009 Aug 21	7	—	—	∴ didn't feed in ~~morning~~ morning. NS 2009 Aug 21	NS
2009 Aug 22	8	All	All	Fed 25µl [artemia] per topsmelt @ 10:15 + 14:30	JR

Cantest Ltd. *Atherinops affinis* **28-d Survival and Growth Test**
Test Observations

Client # & Name: HOW001 & Howe Sound Pulp & Paper. Start Date and Time: 2009 Aug 14 @ 16:45
Sample ID: fe Aug 12, FE End Date: 2009 Aug 27
Analyst(s): NShergill & J. Ross

Date	Day	Conc.	Replicate	Comments (e.g., replicate used for measurements, atypical behaviour of fish, etc.)	Analyst
2009 Aug 22	8	100	100	DO: 8.3 @ 20.3°C aerated 10:53 - 11:13 New DO: 7.5 @ 20.1°C	JR
2009 Aug 22	8	All	All	Water Δ WQ on rep C; completed @ 13:50	JR
2009 Aug 23	9	All	All	Fed 25 µl artemia per hatched fish. @ 0846 +	JR
2009 Aug 23	9	100	All	DO: 8.4 @ 20.5°C aerated 0957 - 1017 NEW DO:	JR
2009 Aug 23	9	All	All	Water Δ completed @ 14:15 WQ rep D	JR
2009 Aug 24	10	All	All	Fed 25 µl artemia/fish @ 0942 & 1730.	Kt/NS
↓	↓	↓	↓	Used Carboy #5. DO = 8.5 @ ~~20~~ 20.9 (we Kt 2009 Aug 24). Aerated	Kt
				from 1552-1612 New DO: 7.7 @ 20.6.	Kt
↓	↓	↓	↓	H_2O Δ @ 1715. WQ rep E	Kt
2009 Aug 25	11	All	All	Fed 25 µl artemia/fish @ 0915 & 1855.	NS
↓	↓	↓	↓	Used Carboy #5. DO: 8.6 @ 20.5 Aerated	
↓	↓	↓	↓	from 1636 to 1656. New DO = 8.1 @ 20.5°C	MG
↓	↓	↓	↓	WQ Rep A. H_2O Δ @ 1730.	
2009 Aug 26	12	All	All	Fed 25 µl artemia/fish @ 1000 & 1328.	NS
2009 Aug 26	12	All	All	Used Carboy #6. DO = 8.5 @ 20.7. Aerated: 1052-1112.	NS
2009 Aug 26	12	All	All	New DO = 8.3 @ 20.5. WQ Rep B. H_2O Δ 1230.	NS
2009 Aug 26	12	50/100	50/100	fish appear weaker than other conc'n	NS
2009 Aug 26	12	—	—	Conc'n without 'B' rep, used Rep C for WQ	NS
2009 Aug 27	13	All	All	fed 25 µl artemia/fish @ 915 & 12:30.	NS
2009 Aug 27	13	-	-	Used carboy #6. DO = 8.9 @ 20.8. Aerated: 958-1018	NS
2009 Aug 27	13	-	-	New DO = 8.4 @ 20.5. H_2O Δ @ 1130. WQ Rep D.	NS
2009 Aug 27	13	SALT CONTROL	A	Salt control - 3 fish were white & dead, surviving fish	NS
2009 Aug 27	13	SALT CONTROL	A	appears normal. Test takedown (too many control	NS
				fish dead).	

NS 2009 Aug 27

CANTEST Ltd. ***Atherinops affinis* Larval Survival and Growth Test**

Daily Test Conditions

Client # & Name: Howe Sound Pulp & Paper Start Date & Time: 2009 Aug 14 @ 16:45

Sample ID: FE Aug 12, FE End Date: 2009 Aug 27

CANTEST #: 908130064, 908200059

Date & Time Sampled: 2009 Aug 12 @ , 2009 Aug 19 @ 930 Analyst(s): J. Ross N Morgill K Tamaki

Date & Time Received: 2009 Aug 13 @ , 2009 Aug 20 @ 957

Sample Measurements: 29.0 NS 2009 Aug 14

Salinity (‰): 31.7 Temp. (°C): 20.0 pH: 6.9 D.O. (mg/L): 7.5

SW Control	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.4	30.3	30.5	30.3	30.5	30.5	30.7
	Final	30.8	30.7	30.5	30.2	30.2	29.9	30.9
Temp. (°C)	Initial	19.6	19.0	19.4	20.1	19.3	19.7	20.1
	Final	19.3	19.0	19.4	19.4	19.6	19.7	19.9
pH	Initial	8.0	7.6	8.0	8.0	7.9	8.0	7.9
	Final	7.7	8.0	8.0	7.9	8.0	8.0	7.9
D.O. (mg/L)	Initial	* ~~9.7~~ 7.7	7.6	7.7	7.7	7.7	7.6	7.8
	Final	(B) ~~8.8~~ 7.4	7.4	7.7	7.7	7.5	7.7	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

(B) WE JR 2009 Aug 15

Salt Control	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	28.4	29.6	28.7	28.3	28.6	28.7	28.8
	Final	29.0	29.9	30.5	30.2	30.2	28.9	29.6
Temp. (°C)	Initial	19.9	19.4	19.0	19.8	20.1	19.6	19.6
	Final	19.4	19.0	19.4	19.4	19.5	19.7	19.9
pH	Initial	8.0	7.6	8.0	8.0	8.0	8.0	8.0
	Final	7.7	8.0	8.0	7.9	8.0	8.0	7.9
D.O. (mg/L)	Initial	7.7	7.7	7.7	7.7	7.7	7.6	7.8
	Final	7.6	7.1	7.7	7.7	7.5	7.5	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

6.25% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.3	30.3	30.4	30.1	30.5	30.6	30.7
	Final	30.9	30.6	30.5	31.0	30.5	30.5	30.9
Temp. (°C)	Initial	19.6	19.2	19.1	19.9	19.2	19.7	19.8
	Final	19.7	19.0	19.4	19.4	19.4	19.7	19.9
pH	Initial	~~7.8~~ 7.7	7.5	7.8	7.7	7.7	7.6	7.6
	Final		7.9	8.0	7.9	8.0	8.0	8.0
D.O. (mg/L)	Initial	* ~~9.7~~ 7.7	7.6	7.7	7.7	7.7	7.7	7.8
	Final	7.4	7.3	7.6	7.7	7.5	7.5	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

12.5% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.2	30.2	30.4	30.1	30.5	30.6	30.7
	Final	31.0	30.6	30.5	31.0	30.5	30.5	30.1
Temp. (°C)	Initial	19.6	19.0	19.4	19.8	19.2	19.7	19.9
	Final	19.6	19.0	19.4	19.5	19.5	19.7	19.9
pH	Initial	7.6	7.4	7.7	7.5	7.9	7.4	7.3
	Final	7.7	8.0	8.0	7.9	8.0	7.9	7.9
D.O. (mg/L)	Initial	* ~~8.3~~ 7.7	7.6	7.7	7.7	7.7	7.7	7.8
	Final	7.5	7.2	7.6	7.7	7.5	7.5	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

CANTEST Ltd. ***Atherinops affinis*** **Larval Survival and Growth Test**

Daily Test Conditions

Client # & Name: Howe Sound Pulp & Paper

Start Date & Time: 2009 Aug 14 @ 16:45

Sample ID: Le Anglo, FE

End Date: 2009 Aug 27

CANTEST #: 908130064, 908200059

25% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.0	30.1	30.3	30.1	30.3	30.6	30.7
	Final	30.6	30.5	30.5	31.0	30.8	30.5	30.9
Temp. (°C)	Initial	19.6	19.2	19.6	19.8	19.4	19.9	19.9
	Final	19.7	19.0	19.3	19.5	19.4	19.7	19.9
pH	Initial	7.4	7.4	7.5	7.3	7.2	7.1	7.0
	Final	7.7	8.0	8.0	7.9	8.0	7.9	8.0
D.O. (mg/L)	Initial	9.3 7.7	7.5	7.7	7.7	7.7	7.7	7.8
	Final	7.3	7.2	7.6	7.6	7.4	7.4	7.5
Analyst		NS JR	JR	JR NS	NS KF	KF NS	NS NS	NS NS

50% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	29.7	30.0	30.0	30.0	30.1	30.5	30.6
	Final	30.2	30.1	30.4	31.0	30.8	30.6	30.6
Temp. (°C)	Initial	19.6	19.7	19.8	19.9	19.5	20.0	20.1
	Final	19.7	19.1	19.4	19.5	19.5	19.6	19.8
pH	Initial	7.2	7.3	7.3	7.1	7.0	6.9	6.9
	Final	7.7	7.9	8.0	7.9	8.0	7.9	8.0
D.O. (mg/L)	Initial *	9.3 7.7	7.4	7.6	7.7	7.7	7.6	7.8
	Final	7.1	7.0	7.5	7.6	7.2	7.3	7.4
Analyst		NS JR	JR	JR NS	NS KF	KF NS	NS NS	NS NS

100% v/v	Day	1	2	3	4	5	6 WE NS 2009 Aug 27	
Salinity (‰)	Initial	29.0	29.4	29.2	29.7	29.7	30.1	30. 29.7
	Final	29.4	29.5	30.4	30.2	30.2	30.5	30.2
Temp. (°C)	Initial	19.5	21.0	20.6	20.0	19.7	20.2	20.5
	Final	19.6	19.1	19.4	19.6	19.4	19.7	19.8
pH	Initial	7.1	7.1	7.1	7.0	6.8	6.7	6.7
	Final	7.7	7.9	8.0	7.9	8.0	7.9	8.0
D.O. (mg/L)	Initial *	9.5 7.7	7.1	7.6	7.7	7.7	7.7	7.8
	Final	6.8	6.6	7.4	7.6	6.9	7.1	7.1
Analyst		NS JR	JR	JR NS	NS KF	KF NS	NS NS	NS NS

Note: See test comments for salinity adjustments, effluent bag used and replicate used for daily measurements

* WE NS 2009 Aug 14 - Did fresh H_2O results then switched to SW.

CANTEST Ltd. ***Atherinops affinis*** **Larval Survival and Growth Test**
Daily Test Conditions

Client # & Name: HDWOOD Halos Sounds Pulp & Paper
Start Date & Time: 2009 Aug 14 @ 16:45
Sample ID: S Aug 13, FE
End Date: 2009 Aug 27
CANTEST #: 908130064, 908200059
Date & Time Sampled: 2009 Aug 12@ , 2009 Aug 19@ 930
Analyst(s): PS Lagerio J. Ross Kitamaki M. Grey
Date & Time Received: 2009 Aug 13@ , 2009 Aug 20@
Sample Measurements:

Salinity (‰): 29.2 Temp. (°C): 20.6 pH: 6.8 D.O. (mg/L): 7.9

SW Control	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.4	29.9	29.9	30.0	31.0	29.9	30.2
	Final	~~19.0~~ 31.0 (B)	30.4	29.8	30	31.1	30.1	
Temp. (°C)	Initial	20.0	19.4	19.9	19.2	19.0	19.7	19.5
	Final	19.0	19.3	19.6	19.8	19.7	19.5	
pH	Initial	7.9	8.0	8.0	8.0	7.9	7.7	7.9
	Final	8.0	8.0	8.0	7.9	7.9	7.7	
D.O. (mg/L)	Initial	7.9	7.4	7.6	7.6	7.8	8.1	7.9
	Final	7.9	~~7.38~~ 7.5 (C)	7.2	7.2	7.3	7.4	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

Salt Control	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	28.4	28.1	28.2	28.5	28.5	28.8	28.4
	Final	27.4	28.6	28.3	29.0	28.7	28.9	
Temp. (°C)	Initial	20.5	19.3	19.7	19.9	19.9	19.9	19.7
	Final	~~20.5~~ 19.2	19.2	19.6	19.7	19.7	19.6	
pH	Initial	7.9	8.0	8.0	8.1	8.2	8.1	8.1
	Final	8.0	8.0	8.0	8.1	8.0	8.0	
D.O. (mg/L)	Initial	7.9	7.5	7.5	7.6	7.7	7.7	7.9
	Final	7.9	~~7.38~~ 7.6 (C)	7.2	7.4	7.2	6.6	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

6.25% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.6	29.9	29.9	30.2	30.6	29.9	30.2
	Final	31.3	30.6	30.3	30.6	30.7	30.4	
Temp. (°C)	Initial	19.9	19.2	19.6	19.3	19.1	19.9	19.7
	Final	19.3	19.1	19.5	19.7	19.6	19.6	
pH	Initial	7.7	7.9	7.9	7.9	7.9	7.6	7.8
	Final	8.0	8.1	8.0	8.0	7.9	7.8	
D.O. (mg/L)	Initial	7.9	7.5	7.7	7.7	7.7	8.1	7.9
	Final	7.8	~~7.28~~ 7.6 (C)	7.2	7.1	6.9	6.9	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

12.5% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.6	30.0	30.0	30.2	30.7	29.9	30.2
	Final	31.1	30.5	30.2	30.5	31.0	30.4	
Temp. (°C)	Initial	19.8	19.2	19.6	19.3	19.0	20.1	19.6
	Final	19.3	19.0	19.5	19.6	19.7	19.5	
pH	Initial *	~~7.7~~ 7.5	7.9	7.8	7.7	7.7	7.4	7.6
	Final	8.0	8.1	8.0	8.0	7.9	7.8	
D.O. (mg/L)	Initial	7.9	7.5	7.7	7.7	7.7	8.2	7.9
	Final	7.7	7.2	7.2	7.2	7.0	7.2	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

*WE NS 2009 Aug 21 (B) WE JR 2009 Aug 22 (C) recalibrated DO meter from fresh to salt water JR 2009 Aug 23

CANTEST Ltd. ***Atherinops affinis*** **Larval Survival and Growth Test**

Daily Test Conditions

Client # & Name: Howool & House Solids Pulp & Paper

Start Date & Time: 2009 Aug 14 @ 1645

Sample ID: Eff Aug 13, FE

End Date: 2009 Aug 27

CANTEST #: 908130004, 908200059

25% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.5	29.9	29.9	30.2	30.7	30.2	30.3
	Final	31.1	30.5	30.2	30.4	30.9	NS 30.6	
Temp. (°C)	Initial	19.9	19.2	19.6	19.7	19.1	19 20.6	19.6
	Final	19.4	19.0	19.5	19.6	19.6	19.5	
pH	Initial	7.1	7.8	7.6	7.6	7.5	7.2	7.4
	Final	8.0	8.1	8.0	8.0	7.9	7.9	
D.O. (mg/L)	Initial	7.9	7.5	7.7	7.7	7.7	8.2	7.9
	Final	7.6	7.2	7.0	7.2	7.0	7.2	
Analyst		NS JR	JR	JR NS	KF MG	MG NS	NS NS	NS

50% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.3	29.8	29.8	30.1	30.4	30.5	30.4
	Final	30.9	30.1	30.1	30.2	30.9	30.4	
Temp. (°C)	Initial	20.1	19.2	19.6	19.6	19.4	20.9	19.8
	Final	19.5	19.0	19.6	19.5	19.6	19.5	
pH	Initial	6.9	7.8	7.5	7.3	7.3	7.0	7.3
	Final	8.0	8.1	8.1	8.0	7.9	7.8	
D.O. (mg/L)	Initial	7.9	7.5	7.5	7.7	7.8	7.8	7.9
	Final	7.4	6.9	7.3	6.9	6.8	6.1	
Analyst		NS JR	JR	JR NS	KF MG	MG NS	NS NS	NS

100% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	29.2	29.5	29.7	29.9	29.8	30.5	30.7
	Final	30.2	29.8	30.1	30.0	30.1	30.7	
Temp. (°C)	Initial	20.1	19.6	19.6	20.4	20.1	20.7	20.2
	Final	19.5	19.0	19.7	19.6	19.7	19.5	
pH	Initial	6.8	7.6	7.4	7.7	7.1	7.0	7.1
	Final	8.0	8.1	7.9	8.0	8.0	7.9	
D.O. (mg/L)	Initial	7.9	7.4	7.5	7.6	7.8	7.8	8.0
	Final	7.3	6.6	7.1	6.8	6.7	6.0	
Analyst		NS JR	JR	JR NS	KF MG	MG NS	NS NS	NS

Drew 2009 Aug 25

Note: See test comments for salinity adjustments, effluent bag used and replicate used for daily measurements

Cantest Ltd.

Atherinops affinis **Larval Survival and Growth Test**
Survival Data

Client Name and Number: Howool HOWE SOUND PULP & PAPER

Start Date & Time: 2009 Aug 14 @ 1655

Organism Lot #: AB090813

Toxcalc ID:

End Date: 2009 Aug 27

Analyst(s): N Shergill J. Ross K. Tomak

Age at Start of Test: 48hrs

Conc. (mg/L)	Date	# Hatched									Total # Surviving								
		2009 Aug15	2009 Aug16	2009 Aug17	2009 Aug18	2009 Aug19	2009 Aug20	2009 Aug21	2009 Aug22	2009 Aug23	2009 Aug15	2009 Aug16	2009 Aug17	2009 Aug18	2009 Aug19	2009 Aug20	2009 Aug21	2009 Aug22	2009 Aug23
	Day	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Control	A	0	0	0	0	0	0	1	4	4	5	5	5	5	5	5	5	5	5
	B	0	0	0	0	0	0	1 WE NS 2009 Aug21	2	2	5	5	5	5	5	4	4	3	3
	C	0	0	0	0	0	0	~~2~~ 0	2	3	5	5	5	5	5	5	5	4	4
	D	0	0	0	0	0	0	2	4	4	5	5	5	5	WE NS 2009 Aug19 ~~4*~~ 5	5	5	5	5
	E	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5	5	5
Salt Control	A	0	0	0	0	0	0	0	3	4	5	5	5	5	5	5	5	5	5
	B	0	0	0	0	0	0	0	2	2	5	5	5	5	5	5	5	5	5
	C	0	0	0	0	0	0	0	0	3	5	4	4*	4	4*	4	4	4	4
	D	0	0	0	0	0	0	0	4	4	5	5	5	5	5	5	5	5	5
	E	0	0	0	0	0	0	0	3	3	5	5	5	5	5	5	5	3	3
6.25	A	0	0	0	0	0	0	0	3	3	5	5	5	5	5	5	5	5	5
	B	0	0	0	0	0	0	1	3	3	5	5	5	5	5	5	5	5	5
	C	0	0	0	0	0	0	0	4	5	5	5	5 •	5	5	5	5	5	5
	D	0	0	0	0	0	0	2	5	5	5	5	5 •	5	5	5	5	5	5
	E	0	0	0	0	0	0	2	4	5	5	5	5	5	5	5	5	5	5
12.5	A	0	0	0	0	0	0	2	3	3	5	5	5	5	5	5	5	4	3
	B	0	0	0	0	0	0	1	4	4	5	5	5 •	5	5	5	5	4	4
	C	0	0	0	0	0	0	0	1	2	5	5	5 •	5	5	3*	3	3	3
	D	0	0	0	0	0	0	0	1	1	5	5	5 •	5	5	5	5	5	5
	E	0	0	0	0	0	0	0	1	3	5	5	5	5	5	5	5	5	5

*2009 Aug20 (NS) – 2 dead eggs attached to live eggs.

#NS 2009 Aug17: one egg has gone white

• NS 2009 Aug 17: eggs do not appear healthy (look clear).

*Salt Control C – has one dead egg attached to others can not remove: NS, 2009 Aug19.

Cantest Ltd.

***Atherinops affinis* Larval Survival and Growth Test**
Survival Data

Client Name and Number Tembec HOUSE SOUND PULP & PAPER

Start Date & Time: 2009 Aug 14 @ 1645

Organism Lot #: AB090813

Toxcalc ID:

End Date:

25	A	0	0	0	0	0	0	N	3	4	5	5	5	5	5	5	5	4	4
	B	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	3	3
	C	0	0	0	0	0	0	0	3	4	5	5	5	5	5	5	5	5	5
	D	0	0	0	0	0	0	1	2	2	5	5	5 WEDS 2009 Aug 19	4	4	4	4	3	3
	E	0	0	0	0	0	0	0	1	5	5	5	5 WEDS 2009 Aug 19	5	5	5	5	5	5
50	A	0	0	0	0	0	0	0	1	3	5	5	5	5	5	5	5	5	4
	B	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	4	2
	C	0	0	0	0	0	0	0	1	4	5	5	5	5	5	5	5	4	4
	D	0	0	0	0	0	0	0	3	4	5	5	5	5	WEDS 2009 Aug 19 4 5	5	5	5	4
	E	0	0	0	0	0	0	0	4	4	5	5	5	5	5	5	5	5	5
100	A	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	3	2	2
	B	0	0	0	0	0	0	0	0	1 (A)	5	5	5	5	5	5	5	5	2
	C	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	5	5
	D	0	0	0	0	0	0	0	0	2	5	5	5	5	5	5	5	5	5
	E	0	0	0	0	0	0	0	0	(B) ~~0~~ 0	5	5	5	5	5	5	5	5	5
Analyst		JR	JR	NS	KT	NS	NS	NS	JR	JR	JR	JR	NS	KT	NS	NS	NS	JR	JR

*see test comments

Note: 50% & 100% difficult to see eggs, (NS 2009 Aug 17) used light table to look @ survival.

(A) - dead/hatching

(B) - WEDS 2009 Aug 23

Cantest Ltd.

Atherinops affinis Larval Survival and Growth Reference Toxicant Test
Survival Data

Client: How001 , howe sound pulp and paper
Organism Lot #: AB090813 Toxcalc ID: n/a
Analyst(s): MBrescacin NShergill, MGrey

Start Date & Time: 2009 Aug 24 @ 1455
End Date: 2009 Aug 27
Age at Start of Test: <24hrs

Conc. (mg/L)	Date	# Hatched 2009 Aug 24	2009 Aug 25	2009 Aug 26	2009 Aug 27						Total # Surviving 2009 Aug 24	2009 Aug 25	2009 Aug 26	2009 Aug 27					
	Day	10	11	12	13	14					10	11	12	13					
Lab	A	4	4								5	4	4	4					
Control	B	2	2								2	2	2	2					
	C	3	3 (B)								3	3	3	3					
	D	4	4								4	4	4	4					
	E	5	5								5	5	5	5					
Salt	A	4	4 (B)								4	4	4	1					
Control	B	2	3								2	3	3	3					
	C	3	3								3	3	3	3					
	D	4	4								4	4	4	4					
	E	3	3								3	3	3	3					
6.25	A	4	B4 (C)								4	3	3	3					
	B	4	4								4	4	4	4					
	C	5	5								5	3	3	3					
	D	5	5								5	5 we MB 5	5	5					
	E	5	5								5	4 5	5	4					
12.5	A	(A) ~~4~~ 3	3								(A) ~~4~~ 3	2	2	2					
	B	4	4								4	4 (B)	2	2					
	C	2	3								3	3	2	2					
	D	1	1								5	3 (B)	1	1					
	E	3	3								4	1 2	3	3					

(A) we MB 2009 Aug 24 (C) we MG 2009 Aug 25, 2009
(B) 1 pale MG

Cantest Ltd.

***Atherinops affinis* Larval Survival and Growth Reference Toxicant Test**
Survival Data

Client: How001 , howe sound pulp and paper

Start Date & Time: 2009 Aug 14 @ 1645

Organism Lot #: AB090813

Toxcalc ID: n/a

End Date: 2009 Aug 27

25	A	4	4								4	3	1	0					
	B	0	0								3	3	0	0					
	C	4	4								5	4	4	4					
	D	2	2								3	2	2	2					
	E	5	5								5	5	5	5					
50	A	3	3								3	3	3	3					
	B	2	2								2	0	0	0					
	C	4	4								4	4	4	3					
	D	4	4								4	4	4	3					
	E	4	4								4	4	4	4					
100	A	2	2								2	2	2	1					
	B	2	2								2	2	2	2					
	C	0	0								5	0	0	0					
	D	2	3								5	3	3	3					
	E	2	4								5	4	4	4					
Analyst		MRB	MB								MRB	MB	NS	NS					

*see test comments

Feeding → No # label = 5 fish
green = 4 fish
black = 3 fish
blue = 2 fish
red = 1 fish

CANTEST Ltd. ***Atherinops affinis*** **Larval Survival and Growth Test**
Survival Data

Client # & Name: Howool Howe Sound Pulp & Paper
Sample ID: Fe Aug 12, FE
CANTEST #: 908130064, 908200059,
ToxCalc. File ID:

Start Date & Time: 2009 Aug 14 @ 1645
End Date: 2009 Aug 27
Organism Lot #: AB090813
Age at Start of Test: 48hr

Analyst(s): NSherzill

		# Surviving Organisms										
Conc. (%v/v)	Replicate #	8	9	10	Day 11	12	13	14	% Survival	% Mortality	% Mean Mortality	SD (%)
	Date						2009 Aug 27					
SW Control	A						4					
	B						2 *					
	C						3					
	D						4 *					
	E						5					
Salt	A						1					
Control	B						3					
	C						3					
	D						4					
	E						3					
6.25	A						~~4~~ 3					
	B						~~3~~ 4					
	C						3					
	D						5 *					
	E						4					
12.5	A						2 *					
	B						2 *					
	C						2 *					
	D						1					
	E						3					
25	A						0					
	B						0					
	C						4 *					
	D						2 *					
	E						5					
50	A						3 *					
	B						0					
	C						3 *					
	D						3 *					
	E						4					
100	A						1					
	B						2					
	C						0					
	D						3					
	E						4					
Analyst							NS					

*see test comments

*1 fish looks weak (NS 2009 Aug 27)
Salt control A dead fish were white.

Cantest Ltd.

Atherinops affinis Survival and Growth Test
Artemia Counts

Client #'s: HAD001

Start Date: 2009 Aug 14 @ 16:45

	Counts											
Date	1	2	3	4	5	6	7	8	9	10	Average	Analyst
2009 Aug 21	20	18	22	20	17	22	15	21	19	13	18	NS
2009 Aug 22	18	18	22	20	20	20	26	23	19	23	21	JR
2009 Aug 23	21	21	27	18	16	20	18	25	18	23	21	JR
2009 Aug 24	36 25	41	43	35	36	39	44	41	44	39	39.8	NS
2009 Aug 25	50 39	28	33	35	36	38	37	39	41	42	~37	NS
2009 Aug 26	45	35	33	41	42	34	37	38	42	40	38.7	NS
2009 Aug 27	36	41	44	46	40	35	42	38	32	40	39.4	NS

Date	Conc. Volume (ml)	Final Volume (ml)	Amount Fed/Fish (µl)	Analyst
2009 Aug 22	250	300	25	JR
2009 Aug 23	300	400	25	JR
2009 Aug 24	250	60	25	KH
2009 Aug 25	200	100	25	NS
2009 Aug 26	100 50	250	25	NS
2009 Aug 27	75	125	25	NS
2009 Aug 28	100	60	25	NS

Note: Day 1 & 2 count 20 artemia / 25ml shot - easier for baby topsmelt to eat.
Day 3 - until testend: count 40 artemia / 25ml shot.

CANTEST Ltd.

Salinity Adjustment Using Dry Salts Datasheet

Client # and Name: CONTROL - Salt

Sample ID: NA

CANTEST ID: NA

Date & Time Sampled : NA

Date & Time Received: NA

Date and Time	Carboy #	Volume Adjusted (L)	pH Before Salt Added	pH After Salt Added	Salinity Before Salt Added (ppt)	Salinity After Salt Added (ppt)	Mass of Salt Added (g)	Balance	Test Used For	Analyst
2009 Aug 13 @ 1430	—	10	6.5	8.0	0	28.4	372.2	003538	Topsmelt	JR
~~2009 Aug 16 @ 1530~~ JRWE 2009 Aug 16										
2009 Aug 23	—	10	6.5	8.2	0	26.5	372.2	003538	Topsmelt	JR
2009 Aug 23	—	10	8.2	8.2	26.5	29.0	+30.5	003538	Topsmelt	JR

Description of Salt: Oceanpure

Salt Lot # or Received Date: 2008 Mar 14

Comments:

CANTEST Ltd.

Salinity Adjustment Using Dry Salts Datasheet

Client # and Name: HOW001 Howe Sound Pulp & Paper Ltd.

Sample ID: Fe Aug 12, Fe (G# 10081333),

CANTEST ID: 908130064, 908200059,

Date & Time Sampled: 2009 Aug 12@0930 , 2009 Aug 19@930,

Date & Time Received: 2009 Aug 13@0830 , 2009 Aug 20@957,

Date and Time	Carboy #	Volume Adjusted (L)	pH Before Salt Added	pH After Salt Added	Salinity Before Salt Added (ppt)	Salinity After Salt Added (ppt)	Mass of Salt Added (g)	Balance	Test Used For	Analyst
2009 Aug 13@1530	1	~10L	6.5	6.6	1.5	29.3	392.8	003538	Topsmelt	JR
2009 Aug 16@1530	2	~10L	6.8	6.7	1.3	29.6	372.2	003538	Topsmelt	JR
2009 Aug 18@1730	3	~10L	6.7	6.6	1.2	29.4	376.2	003538	Topsmelt	KT
2009 Aug 20@1450	4	~10L	6.6	6.4	1.3	29.4	372.1	003538	Topsmelt	JR
2009 Aug 23@1020	5	~10L	6.6	6.6	1.5	29.7	360.0	003538	Topsmelt	JR
2009 Aug 25@715	6	~10L	6.8	6.7	1.5	30.1	360.2	003538	Topsmelt	KT
2009 Aug 27@1405	7	~10L	6.6	6.7	1.0	29.2	380.0	003538	Topsmelt	KT

Description of Salt: Ocean pure Salt Lot # or Received Date: 2008 Mar 14

Comments:

Randomization Chart for Topsmelt Tests

Client Name:
Test Date:

Use the coloured tape to find appropriate concentrations and put beakers back in proper position following daily water change.

Position Map
Back Wall

5	10	15	20	25	30	35
4	9	14	19	24	29	34
3	8	13	18	23	28	33
2	7	12	17	22	27	32
1	6	11	16	21	26	31

Front of Counter

Position #	Treatment	Replicate	Colour
31	SW Control	A	Red
19	SW Control	B	Red
30	SW Control	C	Red
29	SW Control	D	Red
7	SW Control	E	Red
6	Salt Control	A	Blue
18	Salt Control	B	Blue
23	Salt Control	C	Blue
14	Salt Control	D	Blue
32	Salt Control	E	Blue
21	6.25%	A	Yellow
22	6.25%	B	Yellow
27	6.25%	C	Yellow
33	6.25%	D	Yellow
26	6.25%	E	Yellow
16	12.5%	A	Green
1	12.5%	B	Green
13	12.5%	C	Green
3	12.5%	D	Green
8	12.5%	E	Green
24	25.0%	A	Yellow Glo
17	25.0%	B	Yellow Glo
10	25.0%	C	Yellow Glo
34	25.0%	D	Yellow Glo
11	25.0%	E	Yellow Glo
2	50.0%	A	Leaf
12	50.0%	B	Leaf
5	50.0%	C	Leaf
9	50.0%	D	Leaf
35	50.0%	E	Leaf
20	100%	A	White
25	100%	B	White
4	100%	C	White
15	100%	D	White
28	100%	E	White

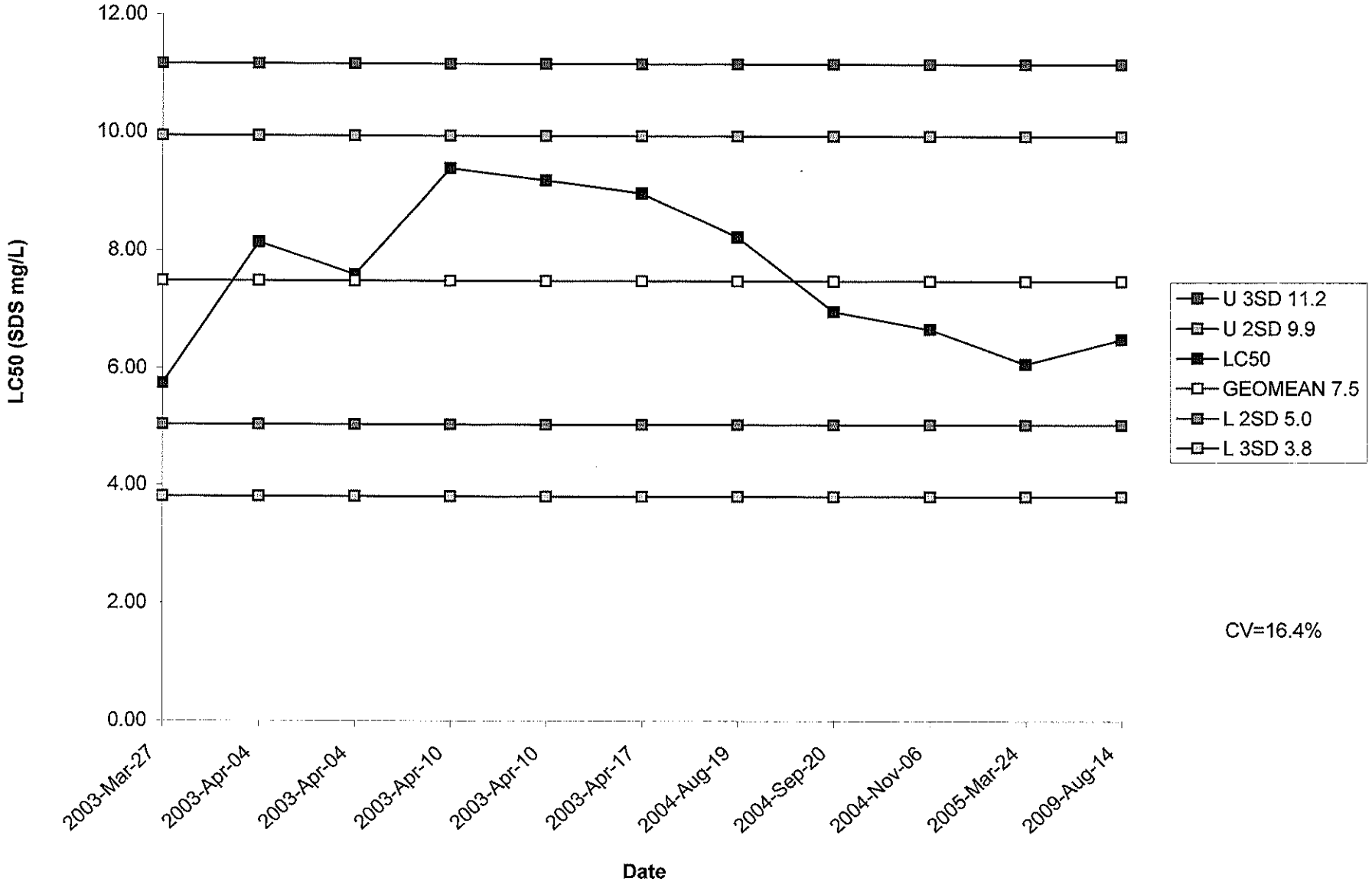
Orange Glo JR 2009Aug13

dark blue JR 2009Aug13

Cantest Ltd.

Atherinops affinis (Topsmelt Eggs)

Survival Reference Toxicant Control Chart for SDS



CANTEST WORK ORDER **Quote#**Group# 100820007

(HOW001) Howe Sound Pulp & Paper Ltd. 3838 Port Mellon Highway Port Mellon, BC V0N 2S0 Att'n: Siew Sim Phone# 1(604)884-2763 Fax# 1(604)884-2178	Received: Aug 20/09 Entered: Aug 20/09 9:57 am by mca # samples: 1 Sampler: # bottles: 3 P.O.# 2009-516 Project: Project# Site: Due Date: Sep 30/09

Bottle Types:	Special Instructions:
3 x CLEAR CARBOYS	**Sample Problem Form attached** high temp

908200059 Matrix: WATER Sampled: 08/19/09 Sample Type: Water COC: 2123869
Id: FE
TOPSMELT

Log-In and labelling checked by:	**Date:**

Invoicing Information	Dept:	61	62	63	64	65	66	67

CHAIN OF CUSTODY RECORD

CANTEST

4606 Canada Way
Burnaby, B.C.
V5G 1K5

Tel: 604.734.7276
Fax: 604.731.2386
Toll Free: 800.665.8566

www.cantest.com

2123869

Client Name:	Howe Sound Pulp & Paper Ltd. Partnership HOW001
Postal Code	V0N 2S0
Street Address (including suite number):	3838 Port Mellon Hwy.
City:	Port Mellon
Telephone:	604 884 2763
Fax:	604 884 2178
E-Mail Address (Required for Electronic Reporting):	Siew.Sim@hspp.ca
Contact Name:	Siew Sim
Sampler's Name:	Siew Sim
Quotation Number:	
Project Number:	
Project Name:	
P.O. Number	2009-J16

Page ____ of ____

RESULTS REQUESTED BY:

30 Day 10 Month 09 Year (09 mc Aug 20)

(Surcharges May Apply)

Special Instructions: ☐ AutoFax ☑ AutoEmail ☐ Return Cooler ☐ Ship Sample Bottles (please specify)

28 Days

3 clear carboy

Sample(s) are from a Drinking Water source servicing multiple households Yes ☐

Group Number	Sample Identification	Date/Time Sampled (D/M/Y & 24hr clock)	Sample Type	Total Metals*	Dissolved Metals*	Field Filtered Metals*	Soil Metals*	pH	Conductivity	TSS	TDS	Alkalinity (total / spec.)	BOD	COD	Coliform, Total & E.coli	Coliform, Fecal	F Cl SO_4 NO_3	Nitrite	Oil & Grease (Total / Mineral)	Oil & Grease (Special Waste)	PCP (Tri, Tetra and Penta)	PCP (Mono and Di)	BETX/VPH	VOC	EPH (not PAH corrected)	PAH	LEPH/HEPH (PAH corrected)	PCB	Asbestos	EEM - Topsmelt				HOLD - DO NOT ANALYZE	Number of Containers
10082000 7																																			
908200059	FE	D: Aug 19 T: 0930	Water																											X					3
LAB		D: T:																												~~Eco-Tox~~ TOPSMELT a/p					
		D: T:																																	
		D: T:																																	
USE		D: T:																																	
		D: T:																																	
		D: T:																																	
ONLY		D: T:																																	
		D: T:																																	
		D: T:																																	
		D: T:																																	

Aug 20, 2009 mc

Leslie-Anne mc Aug 20, 2009

Relinquished by: [signature]	Date Aug 19	Time 1100	Received by:		
Relinquished by:	Date	Time	Received by:		
Method of Shipment: DHL	Waybill No.: 745925659	Received for Lab by: [signature]	Date Aug 20 2009	Time 8:40	
Shipped by: [signature]	Shipment Condition:	Cooler opened by:	Date	Time	

Total Number of Containers:

*Please indicate appropriate regulatory guidelines:

WATER	SOIL
☐ CCME	☐ CCME
☐ BC-CSR	☐ BC-CSR
☐ Other (please specify)	☐ Other (please specify)

FOR LABORATORY USE ONLY

Sample State at Receipt: ☑ Ambient ☐ Cold ☐ Frozen ☐ N/A Comments:

Temperature: 24.6 °C Custody Seal Intact? ☐ Yes ☐ No ☑ n/a Number of Coolers/Shipping Containers: 2

CANTEST WORK ORDER

Quote# Group# 100827037

(HOW001) Howe Sound Pulp & Paper Ltd. 3838 Port Mellon Highway Port Mellon, BC V0N 2S0 Att'n: Siew Sim Phone# 1(604)884-2763 Fax# 1(604)884-2178	Received: Aug 27/09 Entered: Aug 27/09 11:15 am by mca # samples: 1 Sampler: # bottles: 5 P.O.# 2009-516 Project: Project# Site: Due Date: Oct 7/09

#129 400 ml Beakers

Bottle Types:	Special Instructions:
2 x RAW - 1 litre plastic (raw) 3 x CLEAR CARBOYS	**Sample Problem Form attached** high temp

908270108 Matrix: WATER Sampled: 08/26/09 Sample Type: Water COC: 2123810
Id: FE

DAPH-100 TOPSMELT

Log-In and labelling checked by:	Date:

Invoicing Information	Dept:	61	62	63	64	65	66	67

CHAIN OF CUSTODY RECORD

CANTEST

4606 Canada Way
Burnaby, B.C.
V5G 1K5

Tel: 604.734.7276
Fax: 604.731.2386
Toll Free: 800.665.8566

www.cantest.com

2123810

Client Name: Howe Sound Pulp & Paper Ltd. Partnership HOW001

Postal Code: V0N 2S0

Street Address (including suite number): 3838 Port Mellon Hwy.

City: Port Mellon

Telephone: 604 884 2763

Fax: 604 884 2178

E-Mail Address (Required for Electronic Reporting): Siew.Sim@hspp.ca

Contact Name: Siew Sim

Sampler's Name: Siew Sim

Quotation Number:

Project Number:

Project Name:

P.O. Number 2009.516

Page ___ of ___

RESULTS REQUESTED BY: Oct 7, 2009

Day Month Year

(Surcharges May Apply)

Special Instructions: ☐ AutoFax ☑ AutoEmail

☐ Return Cooler ☐ Ship Sample Bottles (please specify)

Sample(s) are from a Drinking Water source servicing multiple households ☐ Yes

Group Number	Sample Identification	Date/Time Sampled (D/M/Y & 24hr clock)	Sample Type	Total Metals*	Dissolved Metals*	Field Filtered Metals*	Soil Metals*	pH	Conductivity	TSS	TDS	Alkalinity (total / spec.)	BOD	COD	Coliform, Total & E.coli	Coliform, Fecal	F Cl SO_4 NO_3	Nitrite	Oil & Grease (Total / Mineral)	Oil & Grease (Special Waste)	PCP (Tri, Tetra and Penta)	PCP (Mono and Di)	BETX/VPH	VOC	EPH (not PAH corrected)	PAH	LEPH/HEPH (PAH corrected)	PCB	Asbestos	DAPH=108, Bif	Ferm retreat Enterococ TOPSMELT			HOLD - DO NOT ANALYZE	Number of Containers
100827037																																			
908270168 LAB USE ONLY	FF	D: Aug 26 T: 0930	Water																											X	X				5
		D: T:																																	
		D: T:																																	
		D: T:																																	
		D: T:																																	
		D: T:																																	
		D: T:																																	
		D: T:																																	
		D: T:																																	
		D: T:																																	

Relinquished by: Date Aug 26 Time 1700 Received by:

Relinquished by: Date Time Received by:

Total Number of Containers:

Method of Shipment: DHL Waybill No.: 740 533764 Received for Lab by: VS Date Aug 27 2009 Time 8:30

Shipped by: Shipment Condition: Cooler opened by: Date Time DHL

*Please indicate appropriate regulatory guidelines:

WATER: ☐ CCME ☐ BC-CSR ☐ Other (please specify)

SOIL: ☐ CCME ☐ BC-CSR ☐ Other (please specify)

FOR LABORATORY USE ONLY

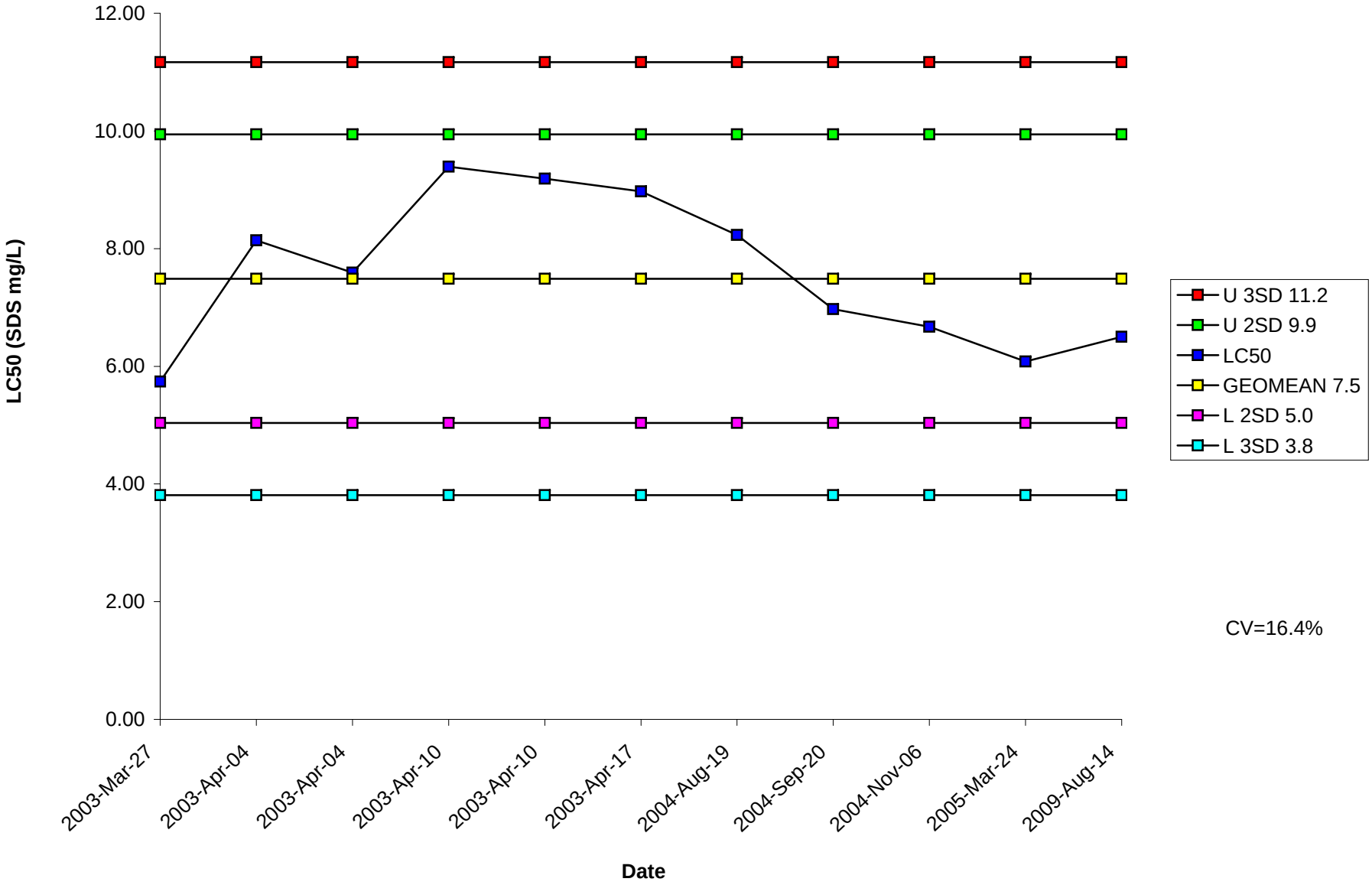
Sample State at Receipt: ☐ Ambient ☐ Cold ☐ Frozen ☐ N/A Comments:

Temperature 6.0°C Custody Seal Intact? ☐ Yes ☐ No ☐ N/A Number of Coolers/Shipping Containers: 1

Cantest Ltd.

Atherinops affinis (Topsmelt Eggs)

Survival Reference Toxicant Control Chart for SDS



CV=16.4%

Howe Sound - 28 day test - Hatching Data
Aug. 2009

		# Hatched													
Conc. (%v/v)	Replicate #	Hatch 1	Hatch 2	Hatch 3	Hatch 4	Hatch 5	Hatch 6	Hatch 7	Hatch 8	Hatch 9	Hatch 10	Hatch 11	% Hatched	% Mean Hatched	SD (%)
	Date	08/15	08/16	08/17	08/18	08/19	08/20	08/21	08/22	08/23	08/24	08/25			
Sea Water Control	A	0	0	0	0	0	0	1	4	4	4	4	80%	72.0%	22.8%
	B	0	0	0	0	0	0	1	2	2	2	2	40%		
	C	0	0	0	0	0	0	0	2	3	3	3	60%		
	D	0	0	0	0	0	0	2	4	4	4	4	80%		
	E	0	0	0	0	0	0	0	4	5	5	5	100%		
Lab Control	A	0	0	0	0	0	0	0	3	4	4	4	80%	68.0%	11.0%
	B	0	0	0	0	0	0	0	2	2	2	3	60%		
	C	0	0	0	0	0	0	0	0	3	3	3	60%		
	D	0	0	0	0	0	0	0	4	4	4	4	80%		
	E	0	0	0	0	0	0	0	3	3	3	3	60%		
6.25	A	0	0	0	0	0	0	0	3	3	4	4	80%	92.0%	11.0%
	B	0	0	0	0	0	0	1	3	3	4	4	80%		
	C	0	0	0	0	0	0	0	4	5	5	5	100%		
	D	0	0	0	0	0	0	2	5	5	5	5	100%		
	E	0	0	0	0	0	0	2	4	5	5	5	100%		
12.5	A	0	0	0	0	0	0	2	3	3	3	3	60%	56.0%	21.9%
	B	0	0	0	0	0	0	1	4	4	4	4	80%		
	C	0	0	0	0	0	0	0	1	2	2	3	60%		
	D	0	0	0	0	0	0	0	1	1	1	1	20%		
	E	0	0	0	0	0	0	0	1	3	3	3	60%		
25	A	0	0	0	0	0	0	2	3	4	4	4	80%	60.0%	40.0%
	B	0	0	0	0	0	0	0	0	0	0	0	0%		
	C	0	0	0	0	0	0	0	3	4	4	4	80%		
	D	0	0	0	0	0	0	1	2	2	2	2	40%		
	E	0	0	0	0	0	0	0	1	5	5	5	100%		
50	A	0	0	0	0	0	0	0	1	3	3	3	60%	68.0%	17.9%
	B	0	0	0	0	0	0	0	0	0	2	2	40%		
	C	0	0	0	0	0	0	0	1	4	4	4	80%		
	D	0	0	0	0	0	0	0	3	4	4	4	80%		
	E	0	0	0	0	0	0	0	4	4	4	4	80%		
100	A	0	0	0	0	0	0	0	0	0	2	2	40%	44.0%	29.7%
	B	0	0	0	0	0	0	0	0	1	2	2	40%		
	C	0	0	0	0	0	0	0	0	0	0	0	0%		
	D	0	0	0	0	0	0	0	0	2	2	3	60%		
	E	0	0	0	0	0	0	0	0	0	2	4	80%		
Analyst		JR	JR	NS	KT	NS	NS	NS	JR	JR	MRB	MG			

	Cumulative % Hatching										
Conc. (%v/v)	1	2	3	4	5	6	7	8	9	10	11
Sea Water Control	0	0	0	0	0	0	16	64	72	72	72
Salt Control	0	0	0	0	0	0	0	48	64	64	68
6.25	0	0	0	0	0	0	20	76	84	92	92
12.5	0	0	0	0	0	0	12	40	52	52	56
25	0	0	0	0	0	0	12	36	60	60	60
50	0	0	0	0	0	0	0	36	60	68	68
100	0	0	0	0	0	0	0	0	12	32	44

Time to complete hatch						Mean	SD
Sea Water Control	8	8	9	8	9	8.4	0.5
Salt Control	9	11	9	8	8	9	1.2
6.25	10	10	9	8	9	9.2	0.8
12.5	8	8	11	8	9	8.8	1.3
25	9		9	8	9	8.75	0.5
50	9	10	9	9	8	9	0.7
100	10	10		11	11	10.5	0.6

Proofed: Ktanaki 2010 Jan 27

Cantest Ltd.

Howe Sound Pulp Paper
Topsmelt 13-day Survival and Growth Test

Survival Data

# Surviving Organisms																	
Conc.	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	%	% Mean	SD
(%v/v)	Date	8/15	8/16	8/17	8/18	8/19	8/20	8/21	8/22	8/23	8/24	8/25	8/26	8/27	Survival	Survival	(%)
Sea Water Control	A	5	5	5	5	5	5	5	5	5	5	4	4	4	80%	72%	23%
	B	5	5	5	5	5	4	4	3	3	2	2	2	2	40%		
	C	5	5	5	5	5	5	5	4	4	3	3	3	3	60%		
	D	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
	E	5	5	5	5	5	5	5	5	5	5	5	5	5	100%		
Salt Control	A	5	5	5	5	5	5	5	5	5	4	4	4	1	20%	56%	22%
	B	5	5	5	5	5	5	5	5	5	2	3	3	3	60%		
	C	5	4	4	4	4	4	4	4	4	3	3	3	3	60%		
	D	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
	E	5	5	5	5	5	5	5	3	3	3	3	3	3	60%		
6.25	A	5	5	5	5	5	5	5	5	5	4	3	3	3	60%	76%	17%
	B	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
	C	5	5	5	5	5	5	5	5	5	5	3	3	3	60%		
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	100%		
	E	5	5	5	5	5	5	5	5	5	5	5	5	4	80%		
12.5	A	5	5	5	5	5	5	5	4	3	3	2	2	2	40%	40%	14%
	B	5	5	5	5	5	5	5	4	4	4	4	2	2	40%		
	C	5	5	5	5	5	3	3	3	3	3	3	2	2	40%		
	D	5	5	5	5	5	5	5	5	5	5	1	1	1	20%		
	E	5	5	5	5	5	5	5	5	5	4	3	3	3	60%		
25	A	5	5	5	5	5	5	5	4	4	4	3	1	0	0%	44%	46%
	B	5	5	5	5	5	5	5	3	3	3	3	0	0	0%		
	C	5	5	5	5	5	5	5	5	5	5	4	4	4	80%		
	D	5	5	5	4	4	4	4	3	3	3	2	2	2	40%		
	E	5	5	5	5	5	5	5	5	5	5	5	5	5	100%		
50	A	5	5	5	5	5	5	5	5	4	3	3	3	3	60%	52%	30%
	B	5	5	5	5	5	5	5	4	2	2	0	0	0	0%		
	C	5	5	5	5	5	5	5	4	4	4	4	4	3	60%		
	D	5	5	5	5	5	5	5	5	4	4	4	4	3	60%		
	E	5	5	5	5	5	5	5	5	5	4	4	4	4	80%		
100	A	5	5	5	5	5	5	3	2	2	2	2	2	1	20%	40%	32%
	B	5	5	5	5	5	5	5	5	2	2	2	2	2	40%		
	C	5	5	5	5	5	5	5	5	5	5	0	0	0	0%		
	D	5	5	5	5	5	5	5	5	5	5	3	3	3	60%		
	E	5	5	5	5	5	5	5	5	5	5	4	4	4	80%		
Analyst		JR	JR	NS	KT	NS	NS	NS	JR	JR	MRB	MG	NS	NS			

Cumulative Mortality													
Conc.	Day												
(%v/v)	1	2	3	4	5	6	7	8	9	10	11	12	13
SW Control	0	0	0	0	0	1	1	3	3	6	7	7	7
Salt Control	0	1	1	1	1	1	1	3	3	9	8	8	11
6.25	0	0	0	0	0	0	0	0	0	2	5	5	6
12.5	0	0	0	0	0	2	2	4	5	6	12	15	15
25	0	0	0	1	1	1	1	5	5	5	8	13	14
50	0	0	0	0	0	0	0	2	6	8	10	10	12
100	0	0	0	0	0	0	2	3	6	6	14	14	15

Percent Cumulative Mortality													
Conc.	Day												
(%v/v)	1	2	3	4	5	6	7	8	9	10	11	12	13
SW Control	0	0	0	0	0	4	4	12	12	24	28	28	28
Salt Control	0	4	4	4	4	4	4	12	12	36	32	32	44
6.25	0	0	0	0	0	0	0	0	0	8	20	20	24
12.5	0	0	0	0	0	8	8	16	20	24	48	60	60
25	0	0	0	4	4	4	4	20	20	20	32	52	56
50	0	0	0	0	0	0	0	8	24	32	40	40	48
100	0	0	0	0	0	0	8	12	24	24	56	56	60

Proofed: KTenaki 2010 Jan 27

Mean and Standard Deviations of Egg and Larvae Survival and Time to Complete Hatching

Concentration	Egg Survival		Larvae Survival		Larvae Growth (mg)		Time to Complete Hatching (days)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sea Water Control (0%)	72.0%	22.8%	72%	23%			8.4	0.5
Salt Control (0%)	68.0%	11.0%	56%	22%			9	1.2
6.25%	92.0%	11.0%	76%	17%			9.2	0.8
12.5%	56.0%	21.9%	40%	14%			8.8	1.3
25.0%	60.0%	40.0%	44%	46%			8.75	0.5
50%	68.0%	17.9%	52%	30%			9	0.7
100%	44.0%	29.7%	40%	32%			10.5	0.6

Larvae Survival on day 13				Mean	SD
Sea Water Control	A	4	80%	72%	23%
	B	2	40%		
	C	3	60%		
	D	4	80%		
	E	5	100%		
Salt Control	A	1	20%	56%	22%
	B	3	60%		
	C	3	60%		
	D	4	80%		
	E	3	60%		
6.25	A	3	60%	76%	17%
	B	4	80%		
	C	3	60%		
	D	5	100%		
	E	4	80%		
12.5	A	2	40%	40%	14%
	B	2	40%		
	C	2	40%		
	D	1	20%		
	E	3	60%		
25	A	0	0%	44%	46%
	B	0	0%		
	C	4	80%		
	D	2	40%		
	E	5	100%		
50	A	3	60%	52%	30%
	B	0	0%		
	C	3	60%		
	D	3	60%		
	E	4	80%		
100	A	1	20%	40%	32%
	B	2	40%		
	C	0	0%		
	D	3	60%		
	E	4	80%		
Analyst		KM			

Egg Survival on day 11				Mean	SD
Sea Water Control	A	4	80%	72.0%	22.8%
	B	2	40%		
	C	3	60%		
	D	4	80%		
	E	5	100%		
Salt Control	A	4	80%	68.0%	11.0%
	B	3	60%		
	C	3	60%		
	D	4	80%		
	E	3	60%		
6.25	A	4	80%	92.0%	11.0%
	B	4	80%		
	C	5	100%		
	D	5	100%		
	E	5	100%		
12.5	A	3	60%	56.0%	21.9%
	B	4	80%		
	C	3	60%		
	D	1	20%		
	E	3	60%		
25	A	4	80%	60.0%	40.0%
	B	0	0%		
	C	4	80%		
	D	2	40%		
	E	5	100%		
50	A	3	60%	68.0%	17.9%
	B	2	40%		
	C	4	80%		
	D	4	80%		
	E	4	80%		
100	A	2	40%	44.0%	29.7%
	B	2	40%		
	C	0	0%		
	D	3	60%		
	E	4	80%		
Analyst		KM			

Proofed: KT 2010 Jan 27

Old report: 2-11-910

Cantest Ltd.

***Atherinops affinis* 28 Day Survival and Growth Test**
Acclimation and Holding Conditions

Eggs Used for Client #'s: Haw001 Date & Time of Arrival: 2009 Aug 13 @ 1130

Organism Lot #: AB090813 Age upon Arrival: <24hrs

Water (L) per Shipping Bag: ~2L Number of Eggs Ordered: 550+55 = 605

Number of Shipping Bags: 1

Arrival Conditions

Bag ID	# Dead	% Dead	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Analyst
~~AB090813~~			29.0	20.9	7.3	OVER	NS

Daily Conditions During Holding/Acclimation

Bag A	Mortalities		Water Quality				
Date	# Dead	% Dead	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Analyst

Bag B	Mortalities		Water Quality				
Date	# Dead	% Dead	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Analyst

Total Daily Mortalities			
Date	# Dead	% Dead	Analyst
Total			

Comments (e.g.egg appearance, acclimation conditions): **Analyst**

2009 Aug 13 - began aerating @ 1131 (took wq first) NS
- 500 mL added @ 1215 NS
- added ~ 4L SW to a clean 20L fish tank, added some overlying water from culture bag, gently poured remaining H_2O + gently pulled strings out of bag + into tank. Airline added. Eggs visible (green) LS

2009 Aug 20: noticed some a couple of topsmelt hatched, NS
WE NS 2009 Aug 20 fed ~3ml of diluted artemia (from Fthm)

AB090813

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel: 970/484-5091 Fax:970/484-2514

ORGANISM HISTORY

DATE: 8/12/09

SPECIES: *Atherinops affinis*

AGE: <24 hour

LIFE STAGE: Embryos

HATCH DATE: Spawned 8/11/09; To hatch 8/16-8/17/09

BEGAN FEEDING: N/A

FOOD: N/A

Water Chemistry Record:	Mean	Range
TEMPERATURE:	23 °C	--
SALINITY/CONDUCTIVITY:	32 ppt	--
TOTAL HARDNESS (as $CaCO_3$):	--	--
TOTAL ALKALINITY (as $CaCO_3$):	182 mg/l	--
pH:	7.76	--

Comments:

Facility Supervisor

Cantest Ltd.

Atherinops affinis **28-d Survival and Growth Test**
Test Observations

Client # & Name: Howood / Howe Sound Pulp & Paper Start Date and Time: 2009 Aug 14 @ 16:45
Sample ID: Fe ~~908200059~~, WE NS 2009 Aug 20 ~~19~~ End Date: 2009 Aug 27
Analyst(s): J. Ross N Shergill K Tomaki WE NS 2009 Aug 20

Date	Day	Conc.	Replicate	Comments (e.g., replicate used for measurements, atypical behaviour of fish, etc.)	Analyst
2009 Aug 14	0	All	All	Used carboy #1 (salinity adjusted). Sample dark	NS
2009 Aug 14	0	All	All	amber, difficult to see through. Warmed up to 19°C.	NS
2009 Aug 14	0	All	All	Did initial WQ. Room lighting - ~~500~~ 50 WE NS 2009 Aug 14 100ft.	NS
2009 Aug 14	0	All	All	finished seeding tests @ 4:45 pm	JR
2009 Aug 15	1	All	All	Used carboy #1 (salinity adjusted). Sample dark amber.	JR
2009 Aug 15	1	All	A	Water quality	JR
2009 Aug 15	1	All	All	Water Δ; all eggs appear normal @ 13:30	JR
2009 Aug 16	2	100	All	Used carboy #1 DO: 8.0 @ 20.9°C aerated 14:06 - 14:31	JR
2009 Aug 16	2	100	All	Sample dark amber and salinity adjusted	JR
2009 Aug 16	2	All	B	Water quality	JR
2009 Aug 16	2	All	All	Water Δ @ 16:00; 1 foggy egg in salt Ctrl C.	JR
2009 Aug 17	3	All	All	Used Carboy #2 (salinity adjusted). Sample dark amber	NS
2009 Aug 17	3	All	All	DO = 7.7 @ 20.0 (100% sample). No Aeration.	NS
2009 Aug 17	3	All	C	Water quality. ~~NS~~ WE, NS 2009 Aug 17	NS
2009 Aug 17	3	All	All	Water Δ @ 1506 . No eggs have hatched yet	NS
2009 Aug 18	4	All	All	Used carboy #2 (salinity Adjusted) Sample dark amber	Kt
↓	4	↓	↓	DO = 7.8 @ 19.8 No aeration required	Kt
↓	4	↓	↓	H_2O Δ @ 1600	Kt
↓	4	D	↓	WQ rep D WE, NS 2009 Aug ~~18~~ 19 WE NS 2009 Aug 19	Kt
2009 Aug 19	5	100%	—	used carboy #~~2~~ 3 (salinity adjusted) sample dark amber.	JR
2009 Aug 19	5	100%	—	~~DO: 7.9 @ 20.5°C aerated 10:35 - 10:40~~ WE, NS 2009 Aug 19 ~~New DO: 7.6 @ 20.2°C~~	JR
2009 Aug 19	5	All	All	DO = 7.6 @ 20.6°C, No Aeration.	NS
2009 Aug 19	5	All	All	H_2O Δ @ 1350 . WQ Rep E.	NS
2009 Aug 20	6	All	All	Used carboy #3 (salinity adjusted). Dark amber sample	NS
2009 Aug 20	6	100%	—	DO = 7.9 @ 20.9°C, No aeration.	NS
2009 Aug 20	6	All	All	H_2O Δ @ 11:10 . WQ Rep A.	NS
2009 Aug 21	7	All	All	Used Carboy #4 (salinity adjusted - new sample)	NS
2009 Aug 21	7	100%	—	Dark amber sample. DO = 7.9 @ 20.6. No Aeration	NS
2009 Aug 21	7	All	All	H_2O Δ @ 1355 . WQ Rep B. Concn of 50%	NS
2009 Aug 21	7	50/100	All	& 100% all reps look smothered by fluffy	NS
2009 Aug 21	7	50/100	All	stuff in sample (debris?). Fed @ 1630, 25ul	NS
2009 Aug 21	7	—	—	per topsmelt hatched. (Note: unhatched in morning	NS
2009 Aug 21	7	—	—	so didn't feed in ~~morning~~ NS 2009 Aug 21) morning.	NS
2009 Aug 22	8	All	All	Fed 25ul [artemia] per topsmelt @ 10:15 + 14:30	JR

Cantest Ltd. *Atherinops affinis* **28-d Survival and Growth Test**
Test Observations

Client # & Name: Hawo01 & Howe Sound Pulp & Paper. Start Date and Time: 2009 Aug 14 @ 16:45
Sample ID: fe Aug 12, FE End Date: 2009 Aug 27
Analyst(s): NShergill & J. Ross

Date	Day	Conc.	Replicate	Comments (e.g., replicate used for measurements, atypical behaviour of fish, etc.)	Analyst
2009 Aug 22	8	100	100	DO: 8.3 @ 20.3°C aerated 10:53 - 11:13 New DO: 7.5 @ 20.1°C	JR
2009 Aug 22	8	All	All	Water Δ. WQ on rep C; completed @ 13:50	JR
2009 Aug 23	9	All	All	Fed 25 µl artemia per hatched fish. @ 0846 +	JR
2009 Aug 23	9	100	All	DO: 8.4 @ 20.5°C aerated 0957 - 1017 NEW DO:	JR
2009 Aug 23	9	All	All	Water Δ completed @ 14:15 WQ rep D	JR
2009 Aug 24	10	All	All	Fed 25 µl artemia/fish @ 0942 & 1730.	Kt/NS
↓	↓	↓	↓	Used Carboy #5. DO = 8.5 @ ~~20~~ 20.9. Aerated	Kt
				from 1552-1612 New DO: 7.7 @ 20.6.	Kt
				H_2O Δ @ 1715. WQ rep E	Kt
2009 Aug 25	11	All	All	Fed 25 µl artemia/fish @ 0915 & 1855.	NS
↓	↓	↓	↓	Used Carboy #5. DO: 8.6 @ 20.5 Aerated	
				from 1636 to 1656. New DO: 8.1 @ 20.5°C	MG
				WQ Rep A. H_2O Δ @ 1730.	
2009 Aug 26	12	All	All	Fed 25 µl artemia/fish @ 1000 & 1328.	NS
2009 Aug 26	12	All	All	Used Carboy #6. DO = 8.5 @ 20.7. Aerated: 1052-1112.	NS
2009 Aug 26	12	All	All	New DO = 8.3 @ 20.5. WQ Rep B. H_2O Δ 1230.	NS
2009 Aug 26	12	50/100	50/100	fish appear weaker than other conc'n	NS
2009 Aug 26	12	—	—	Conc'n without 'B' rep, used Rep C for WQ	NS
2009 Aug 27	13	All	All	fed 25 µl artemia/fish @ 915 & 12:30	NS
2009 Aug 27	13	—	—	Used carboy #6. DO = 8.9 @ 20.8. Aerated: 958-1018	NS
2009 Aug 27	13	—	—	New DO = 8.4 @ 20.5. H_2O Δ @ 1130. WQ Rep D.	NS
2009 Aug 27	13	SALT CONTROL	A	Salt control - 3 fish were white & dead, surviving fish	NS
2009 Aug 27	13	SALT CONTROL	A	appears normal. Test takedown (too many control	NS
				fish dead).	

NS 2009 Aug 27

CANTEST Ltd. ***Atherinops affinis*** **Larval Survival and Growth Test**

Daily Test Conditions

Client # & Name: HW 001 Howe Sound Pulp & Paper Start Date & Time: 2009 Aug 14 @ 16:45

Sample ID: FE Aug 12, FE End Date: 2009 Aug 27

CANTEST #: 908130064, 908200059

Date & Time Sampled: 2009 Aug 12 @ , 2009 Aug 19 @ 930 Analyst(s): J. Ross N Shergill

Date & Time Received: 2009 Aug 13 @ , 2009 Aug 20 @ 957 K Tamaki

Sample Measurements: 29.0 NS 2009 Aug 14

Salinity (‰): 31.7 Temp. (°C): 20.0 pH: 6.9 D.O. (mg/L): 7.5

SW Control	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.4	30.3	30.5	30.3	30.5	30.5	30.7
	Final	30.8	30.7	30.5	30.2	30.7	29.9	30.9
Temp. (°C)	Initial	19.6	19.0	19.4	20.1	19.3	19.7	20.1
	Final	19.3	19.0	19.4	19.4	19.6	19.7	19.9
pH	Initial	8.0	7.6	8.0	8.0	7.9	8.0	7.9
	Final	7.7	8.0	8.0	7.9	8.0	8.0	7.9
D.O. (mg/L)	Initial *	9.7 7.7	7.6	7.7	7.7	7.7	7.6	7.8
	Final (B)	8.8 7.4	7.4	7.7	7.7	7.5	7.7	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

(B) WE JR 2009 Aug 15

Salt Control	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	28.4	29.6	28.7	28.3	28.6	28.7	28.8
	Final	29.0	29.9	30.5	30.2	30.7	29.9	29.6
Temp. (°C)	Initial	19.9	19.4	19.0	19.8	20.1	19.6	19.6
	Final	19.4	19.0	19.4	19.4	19.5	19.7	19.9
pH	Initial	8.0	7.6	8.0	8.0	8.0	8.0	8.0
	Final	7.7	8.0	8.0	7.9	8.0	8.0	7.9
D.O. (mg/L)	Initial	7.7	7.7	7.7	7.7	7.7	7.6	7.8
	Final	7.6	7.1	7.7	7.7	7.5	7.5	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

6.25% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.3	30.3	30.4	30.1	30.5	30.6	30.7
	Final	30.9	30.6	30.5	31.0	30.5	30.5	30.9
Temp. (°C)	Initial	19.6	19.2	19.1	19.9	19.2	19.7	19.8
	Final	19.7	19.0	19.4	19.4	19.4	19.7	19.9
pH	Initial	7.8	7.5	7.8	7.7	7.7	7.6	7.6
	Final	7.7	7.9	8.0	7.9	8.0	8.0	8.0
D.O. (mg/L)	Initial *	9.7 7.7	7.6	7.7	7.7	7.7	7.7	7.8
	Final	7.4	7.3	7.6	7.7	7.5	7.5	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

12.5% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.2	30.2	30.4	30.1	30.5	30.6	30.7
	Final	31.0	30.6	30.5	31.0	30.5	30.5	30.1
Temp. (°C)	Initial	19.6	19.0	19.4	19.8	19.2	19.7	19.9
	Final	19.6	19.0	19.4	19.5	19.5	19.7	19.9
pH	Initial	7.6	7.4	7.7	7.5	7.9	7.4	7.3
	Final	7.7	8.0	8.0	7.9	8.0	7.9	7.9
D.O. (mg/L)	Initial *	9.3 7.7	7.6	7.7	7.7	7.7	7.7	7.8
	Final	7.5	7.2	7.6	7.7	7.5	7.5	7.5
Analyst		NS JR	JR	JR NS	NS KT	KT NS	NS NS	NS NS

CANTEST Ltd. ***Atherinops affinis* Larval Survival and Growth Test**

Daily Test Conditions

Client # & Name: HAW001 Howe Sound Pulp & Paper Start Date & Time: 2009 Aug 14 @ 1645

Sample ID: Se Aug 13, FE End Date: 2009 Aug 27

CANTEST #: 908135064, 908200059

25% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	30.0	30.1	30.3	30.1	30.3	30.6	30.7
	Final	30.6	30.5	30.5	31.0	30.8	30.5	30.8
Temp. (°C)	Initial	19.6	19.2	19.6	19.8	19.4	19.9	19.9
	Final	19.7	19.0	19.3	19.5	19.4	19.7	19.9
pH	Initial	7.4	7.4	7.5	7.3	7.2	7.1	7.0
	Final	7.7	8.0	8.0	7.9	8.0	7.9	8.0
D.O. (mg/L)	Initial	9.3 7.7	7.5	7.7	7.7	7.7	7.7	7.8
	Final	7.3	7.2	7.6	7.6	7.4	7.4	7.5
Analyst		NS JR	JR	JR NS	NS IK	IK NS	NS NS	NS NS

50% v/v	Day	1	2	3	4	5	6	7
Salinity (‰)	Initial	29.7	30.0	30.0	30.0	30.1	30.5	30.6
	Final	30.2	30.1	30.4	31.0	30.8	30.6	30.6
Temp. (°C)	Initial	19.6	19.7	19.8	19.9	19.5	20.0	20.1
	Final	19.7	19.1	19.4	19.5	19.5	19.6	19.8
pH	Initial	7.7	7.3	7.3	7.1	7.0	6.9	6.8
	Final	7.9	7.9	8.0	7.9	8.0	7.9	8.0
D.O. (mg/L)	Initial *	9.3 7.7	7.4	7.6	7.7	7.7	7.6	7.8
	Final	7.1	7.0	7.5	7.6	7.2	7.3	7.4
Analyst		NS JR	JR	JR NS	NS IK	IK NS	NS NS	NS NS

100% v/v	Day	1	2	3	4	5	6 WE NS	2009 Aug 27
Salinity (‰)	Initial	29.0	29.4	29.2	29.7	29.7	30.1	30. 29.7
	Final	29.4	29.5	30.4	30.2	30.2	30.5	30.2
Temp. (°C)	Initial	19.5	21.0	20.6	20.0	19.7	20.2	20.5
	Final	19.6	19.1	19.4	19.6	19.4	19.7	19.8
pH	Initial	7.1	7.1	7.1	7.0	6.8	6.7	6.7
	Final	7.7	7.9	8.0	7.9	8.0	7.9	8.0
D.O. (mg/L)	Initial *	9.5 7.7	7.1	7.6	7.7	7.7	7.7	7.8
	Final	6.8	6.6	7.4	7.6	6.9	7.1	7.1
Analyst		NS JR	JR	JR NS	NS IK	IK NS	NS NS	NS NS

Note: See test comments for salinity adjustments, effluent bag used and replicate used for daily measurements

* WE NS 2009 Aug 14 - Did fresh H_2O results mean switched to SW.

CANTEST Ltd. ***Atherinops affinis*** **Larval Survival and Growth Test**

Daily Test Conditions

Client # & Name: Howood Halde Souuds Pulp & Paper

Start Date & Time: 2009 Aug 14 @ 16:45

Sample ID: Le Aug 12, FE

End Date: 2009 Aug 27

CANTEST #: 908130064, 908200059

Date & Time Sampled: 2009 Aug 12 @ , 2009 Aug 19 @ 9:30

Analyst(s): DS JRoss Kitamaki

Date & Time Received: 2009 Aug 13 @ , 2009 Aug 20 @

Sample Measurements:

Salinity (‰): 29.2 Temp. (°C): 20.6 pH: 6.8 D.O. (mg/L): 7.9

SW Control	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.4	29.9	29.9	30.0	31.0	29.9	30.2
	Final	~~19.0~~ 31.0	30.4	29.8	30	31.1	30.1	
Temp. (°C)	Initial	20.0	19.4	19.9	19.2	19.0	19.7	19.5
	Final	19.0	19.3	19.6	19.8	19.7	19.5	
pH	Initial	7.9	8.0	8.0	8.0	7.9	7.7	7.9
	Final	8.0	8.0	8.0	7.9	7.9	7.7	
D.O. (mg/L)	Initial	7.9	7.4	7.6	7.6	7.8	8.1	7.9
	Final	7.9	~~7.38~~ 5©	7.2	7.2	7.3	7.4	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

Salt Control	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	28.4	28.1	28.2	28.5	28.5	28.8	28.4
	Final	29.4	28.6	28.3	29.0	28.7	28.9	
Temp. (°C)	Initial	20.5	19.3	19.7	19.9	19.9	19.9	19.7
	Final	~~20.5~~ 19.2	19.2	19.6	19.7	19.7	19.6	
pH	Initial	7.9	8.0	8.0	8.1	8.2	8.1	8.1
	Final	8.0	8.0	8.0	8.1	8.0	8.0	
D.O. (mg/L)	Initial	7.9	7.5	7.5	7.6	7.7	7.7	7.9
	Final	7.9	~~7.38~~ 6©	7.2	7.4	7.2	6.6	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

6.25% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.6	29.9	29.9	30.2	30.6	29.9	30.2
	Final	31.3	30.6	30.3	30.6	30.7	30.4	
Temp. (°C)	Initial	19.9	19.2	19.6	19.3	19.1	19.9	19.7
	Final	19.3	19.1	19.5	19.7	19.6	19.6	
pH	Initial	7.7	7.9	7.9	7.9	7.9	7.6	7.8
	Final	8.0	8.1	8.0	8.0	7.9	7.8	
D.O. (mg/L)	Initial	7.9	7.5	7.7	7.7	7.7	8.1	7.9
	Final	7.8	~~7.28~~ 6©	7.2	7.1	6.9	6.9	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

12.5% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.6	30.0	30.0	30.2	30.7	29.9	30.2
	Final	31.1	30.5	30.2	30.5	31.0	30.4	
Temp. (°C)	Initial	19.8	19.2	19.6	19.3	19.0	20.1	19.6
	Final	19.3	19.0	19.5	19.6	19.7	19.5	
pH	Initial *	~~7.7~~ 7.5	7.9	7.8	7.7	7.7	7.4	7.6
	Final	8.0	8.1	8.0	8.0	7.9	7.8	
D.O. (mg/L)	Initial	7.9	7.5	7.7	7.7	7.7	8.2	7.9
	Final	7.7	7.2	7.2	7.2	7.0	7.2	
Analyst		NS JR	JR	JR NS	KT MG	MG NS	NS NS	NS

* WE NS 2009 Aug 21 (B) WE JR 2009 Aug 22 (C) recalibrated DO meter from fresh to salt water JR 2009 Aug 23

CANTEST Ltd. *Atherinops affinis* **Larval Survival and Growth Test**

Daily Test Conditions

Client # & Name: Howool & Howe Sound Pulp & Paper

Start Date & Time: 2009 Aug 14 @ 1645

Sample ID: Fe Aug 12, FE

End Date: 2009 Aug 27

CANTEST #: 908130064, 908200059

25% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.5	29.9	29.9	30.2	30.7	30.2	30.3
	Final	31.1	30.5	30.2	30.4	30.9	NS 30.6	
Temp. (°C)	Initial	19.9	19.2	19.6	19.4	19.1	2009 19 20.6	19.6
	Final	19.4	19.0	19.5	19.6	19.6	Aug 26 19.5	
pH	Initial	7.1	7.8	7.6	7.6	7.5	7.2	7.4
	Final	8.0	8.1	8.0	8.0	7.9	7.8	
D.O. (mg/L)	Initial	7.9	7.5	7.7	7.7	7.7	8.2	7.9
	Final	7.6	7.2	7.0	7.2	7.0	7.2	
Analyst		NS JR	JR	JR NS	KF MG	MG NS	NS NS	NS

50% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	30.3	29.8	29.8	30.1	30.4	30.5	30.4
	Final	30.9	30.1	30.1	30.2	30.9	30.4	
Temp. (°C)	Initial	20.1	19.2	19.6	19.6	19.4	20.9	19.8
	Final	19.5	19.0	19.6	19.5	19.6	19.5	
pH	Initial	6.9	7.8	7.5	7.3	7.3	7.0	7.3
	Final	8.0	8.1	8.1	8.0	7.9	7.8	
D.O. (mg/L)	Initial	7.9	7.5	7.5	7.7	7.8	7.8	7.9
	Final	7.4	6.9	7.3	6.9	6.8	6.1	
Analyst		NS JR	JR	JR NS	KF MG	MG NS	NS NS	NS

100% v/v	Day	8	9	10	11	12	13	14
Salinity (‰)	Initial	29.2	29.5	29.7	29.9	29.8	30.5	30.7
	Final	30.2	29.8	30.1	30.0	30.1	30.7	
Temp. (°C)	Initial	20.1	19.6	19.6	20.4	20.1	20.7	20.2
	Final	19.5	19.0	19.7	19.6	19.7	19.5	
pH	Initial	6.8	7.6	7.4	7.2	~~7.3~~ 7.1	7.0	7.1
	Final	8.0	8.1	7.9	8.0	8.0	7.8	
D.O. (mg/L)	Initial	7.9	7.4	7.5	7.6	7.8	7.8	8.0
	Final	7.3	6.6	7.1	6.8	6.7	6.0	
Analyst		NS JR	JR	JR NS	KF MG	MG NS	NS NS	NS

DWeMG 2009 Aug 25

Note: See test comments for salinity adjustments, effluent bag used and replicate used for daily measurements

Cantest Ltd.

***Atherinops affinis* Larval Survival and Growth Test**
Survival Data

Client Name and Number: Howe Sound Pulp & Paper (Howe Sound Pulp & Paper)
Organism Lot #: AB090813
Toxcalc ID:
Analyst(s): Abigail S. J. Ross K. Tomaki

Start Date & Time: 2009 Aug 14 @ 1655
End Date: 2009 Aug 27
Age at Start of Test: 48hrs

Conc. (mg/L)		# Hatched									Total # Surviving								
	Date	2009 Aug 15	2009 Aug 16	2009 Aug 17	2009 Aug 18	2009 Aug 19	2009 Aug 20	2009 Aug 21	2009 Aug 22	2009 Aug 23	2009 Aug 15	2009 Aug 16	2009 Aug 17	2009 Aug 18	2009 Aug 19	2009 Aug 20	2009 Aug 21	2009 Aug 22	2009 Aug 23
	Day	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Control	A	0	0	0	0	0	0	1	4	4	5	5	5	5	5	5	5	5	5
	B	0	0	0	0	0	0	1 (WE NS 2009 Aug 21)	2	2	5	5	5	5	5	4	4	3	3
	C	0	0	0	0	0	0	2	2	3	5	5	5	5	5	5	5	4	4
	D	0	0	0	0	0	0	2	4	4	5	5	5	5 (WE NS 2009 Aug 19)	4*	5	5	5	5
	E	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5	5	5
Salt	A	0	0	0	0	0	0	0	3	4	5	5	5	5	5	5	5	5	5
Control	B	0	0	0	0	0	0	0	2	2	5	5	5	5	5	5	5	5	5
	C	0	0	0	0	0	0	0	0	3	5	4	4*	4	4*	4	4	4	4
	D	0	0	0	0	0	0	0	4	4	5	5	5	5	5	5	5	5	5
	E	0	0	0	0	0	0	0	3	3	5	5	5	5	5	5	5	3	3
6.25	A	0	0	0	0	0	0	0	3	3	5	5	5	5	5	5	5	5	5
	B	0	0	0	0	0	0	1	3	3	5	5	5	5	5	5	5	5	5
	C	0	0	0	0	0	0	0	4	5	5	5	5*	5	5	5	5	5	5
	D	0	0	0	0	0	0	2	5	5	5	5	5*	5	5	5	5	5	5
	E	0	0	0	0	0	0	2	4	5	5	5	5	5	5	5	5	5	5
12.5	A	0	0	0	0	0	0	2	3	3	5	5	5	5	5	5	5	4	3
	B	0	0	0	0	0	0	1	4	4	5	5	5*	5	5	5	5	4	4
	C	0	0	0	0	0	0	0	1	2	5	5	5*	5	5	3*	3	3	3
	D	0	0	0	0	0	0	0	1	1	5	5	5*	5	5	5	5	5	5
	E	0	0	0	0	0	0	0	1	3	5	5	5	5	5	5	5	5	5

* 2009 Aug 20 (NS) – 2 dead eggs attached to live eggs.

#PS 2009 Aug 17: one eggs has gone white
•NS 2009 Aug 17: eggs also not appear healthy (look clear).

*Salt Control C – has one dead egg attached to others can not remove: NS, 2009 Aug 19.

Cantest Ltd.

Atherinops affinis **Larval Survival and Growth Test**
Survival Data

Client Name and Number HOWE SOUND PULP HOWOOI & PAPER

Start Date & Time: 2009 Aug 14 @ 1645

Organism Lot #: AB090813

Toxcalc ID:

End Date:

25	A	0	0	0	0	0	0	2	3	4	5	5	5	5	5	5	5	4	4
	B	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	3	3
	C	0	0	0	0	0	0	0	3	4	5	5	5	5	5	5	5	5	5
	D	0	0	0	0	0	0	1	2	2	5	5	5	4	4	4	4	3	3
	E	0	0	0	0	0	0	0	1	5	5	5	5	5	5	5	5	5	5
50	A	0	0	0	0	0	0	0	1	3	5	5	5	5	5	5	5	5	4
	B	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	4	2
	C	0	0	0	0	0	0	0	1	4	5	5	5	5	5	5	5	4	4
	D	0	0	0	0	0	0	0	3	4	5	5	5	5	5	5	5	5	4
	E	0	0	0	0	0	0	0	4	4	5	5	5	5	5	5	5	5	5
100	A	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	3	2	2
	B	0	0	0	0	0	0	0	0	1 (A)	5	5	5	5	5	5	5	5	2
	C	0	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	5	5
	D	0	0	0	0	0	0	0	0	2	5	5	5	5	5	5	5	5	5
	E	0	0	0	0	0	0	0	0	(B) ~~5~~ 0	5	5	5	5	5	5	5	5	5
Analyst		JR	JR	NS	Kt	NS	NS	NS	JR	JR	JR	JR	NS	Kt	NS	NS	NS	JR	JR

*see test comments

Note: 50% & 100% difficult to see eggs, (NS 2009 Aug 17) used light table to look @ survival.

(A) - dead hatchling

(B) - WE OR 2009 Aug 23

Cantest Ltd.

***Atherinops affinis* Larval Survival and Growth Reference Toxicant Test**
Survival Data

Client: How001 , howe sound pulp and paper
Organism Lot #: AB090813
Toxcalc ID:
Analyst(s): MBrescacin

Start Date & Time: 2009 Aug 14@1655
End Date: 2009 Aug 27
Age at Start of Test: <24hrs

Conc. (mg/L)	Date	# Hatched									Total # Surviving								
	Date	2009 Aug24	2009 Aug25	2009 Aug26	2009 Aug27						2009 Aug24	2009 Aug25	2009 Aug26	2009 Aug27					
	Day	10	11	12	13	14					10	11	12	13					
Lab	A	4	4								5	4	4	4					
Control	B	2	2								2	2	2	2					
	C	3	3 (B)								3	3	3	3					
	D	4	4								4	4	4	4					
	E	5	5								5	5	5	5					
Salt	A	4	4 (B)								4	4	4	1					
Control	B	2	3								2	3	3	3					
	C	3	3								3	3	3	3					
	D	4	4								4	4	4	4					
	E	3	3								3	3	3	3					
6.25	A	4	~~3~~ 4 (C)								4	3	3	3					
	B	4	4								4	4	4	4					
	C	5	5								5	3	3	3					
	D	5	5								5	5 WE INS 2009 Aug 25	5	5					
	E	5	5								5	~~4~~ 5	5	4					
12.5	A	(A) ~~4~~ 3	3								(A) ~~4~~ 3	2	2	2					
	B	4	4								4	4 (B)	2	2					
	C	2	3								3	3	2	2					
	D	1	1								5	3 (C)	1	1					
	E	3	3								4	1	3	3					

NS 2009 Aug 27

NS 2009 Aug 27

(A) we MBB 2009 Aug24 (C) we MG 2009 Aug25, 2009
(B) 1 pale MG

Cantest Ltd.

Atherinops affinis Larval Survival and Growth Reference Toxicant Test
Survival Data

Client: How001 , howe sound pulp and paper

Start Date & Time: 2009 Aug 14 @ 1645

Organism Lot #: AB090813

Toxcalc ID:

End Date: 2009 Aug 27

25	A	4	4								4	3	1	0					
	B	0	0								3	3	0	0					
	C	4	4								5	4	4	4					
	D	2	2								3	2	2	2					
	E	5	5								5	5	5	5					
50	A	3	3								3	3	3	3					
	B	2	2								3	0	0	0					
	C	4	4								4	4	4	3					
	D	4	4								4	4	4	3					
	E	4	4								4	4	4	4					
100	A	2	2								2	2	2	1					
	B	2	2								2	2	2	2					
	C	0	0								5	0	0	0					
	D	2	3								5	3	3	3					
	E	2	4								5	4	4	4					
Analyst		MRB	MK								MRB	MK	NS	NS					

*see test comments

Feeding → No # label = 5 fish
green = 4 fish
black = 3 fish
blue = 2 fish
red = 1 fish

CANTEST Ltd.

***Atherinops affinis* Larval Survival and Growth Test**
Survival Data

Client # & Name: HOWOOL HOWE SOUND PULP & PAPER
Sample ID: Fe Aug 12, FE
CANTEST #: 908130064, 908200059,
ToxCalc. File ID:

Start Date & Time: 2009 Aug 14 @ 1645
End Date: 2009 Aug 27
Organism Lot #: AB090813
Age at Start of Test: 48hr

Analyst(s): NSherzill

		# Surviving Organisms										
Conc. (%v/v)	Replicate #	8	9	10	Day 11	12	13	14	% Survival	% Mortality	% Mean Mortality	SD (%)
	Date						2009 Aug 27					
SW Control	A						4					
	B						2 *					
	C						3					
	D						4 *					
	E						5					
Salt Control	A						1					
	B						3					
	C						3					
	D						4					
	E						3					
6.25	A						~~4~~ 3					
	B						~~3~~ 4					
	C						3					
	D						5 *					
	E						4					
12.5	A						2 *					
	B						2 *					
	C						2 *					
	D						1					
	E						3					
25	A						0					
	B						0					
	C						4 *					
	D						2 *					
	E						5					
50	A						3 *					
	B						0					
	C						3 *					
	D						3 *					
	E						4					
100	A						1					
	B						2					
	C						0					
	D						3					
	E						4					
Analyst							NS					

*see test comments

*1 fish looks weak (NS 2009 Aug 27)
Salt control A dead fish were white.

Cantest Ltd.

Atherinops affinis Survival and Growth Test
Artemia Counts

Client #'s: HA0001

Start Date: 2009 Aug 14 @ 16:45

	Counts											
Date	1	2	3	4	5	6	7	8	9	10	Average	Analyst
2009 Aug 21	20	18	22	29	27	22	15	21	19	13	18	NS
2009 Aug 22	18	18	22	20	20	20	26	23	19	23	21	JR
2009 Aug 23	21	21	27	18	16	20	18	25	18	23	21	JR
2009 Aug 24	36	41	43	35	36	39	44	41	44	39	39.8	NS
2009 Aug 25	50 39	28	33	35	36	38	37	39	41	42	~37	NS
2009 Aug 26	45	35	33	41	42	34	37	38	42	40	38.7	NS
2009 Aug 27	36	41	44	46	40	35	42	38	32	40	39.4	NS

Date	Conc. Volume (ml)	Final Volume (ml)	Amount Fed/Fish (µl)	Analyst
2009 Aug 21	250	300	25	JR
2009 Aug 23	300	400	25	JR
2009 Aug 24	250	60	25	KH
2009 Aug 25	200	100	25	NS
2009 Aug 26	100	50	25	NS
2009 Aug 27	75	125	25	NS
2009 Aug 28	100	60	25	NS

Note: Day 1 & 2 count 20 artemia / 25ul shot - easier for baby topsmelt to eat.
Day 3 - until testend: count 40 artemia / 25ul shot.

CANTEST Ltd.

Salinity Adjustment Using Dry Salts Datasheet

Client # and Name: CONTROL - Salt

Sample ID: NA

CANTEST ID: NA

Date & Time Sampled : NA

Date & Time Received: NA

Date and Time	Carboy #	Volume Adjusted (L)	pH Before Salt Added	pH After Salt Added	Salinity Before Salt Added (ppt)	Salinity After Salt Added (ppt)	Mass of Salt Added (g)	Balance	Test Used For	Analyst
2009 Aug 13 @ 1430	—	10	6.5	8.0	0	28.4	372.2	003538	Topsmelt	JR
~~2009 Aug 16 1530~~ JR WE 2009 Aug 16										
2009 Aug 23	—	10	6.5	8.2	0	26.5	372.2	003538	Topsmelt	JR
2009 Aug 23	—	10	8.2	8.2	26.5	29.0	+30.5	003538	Topsmelt	JR

Description of Salt: Oceanpure Salt Lot # or Received Date: 2008 Mar 14

Comments:

CANTEST Ltd.

Salinity Adjustment Using Dry Salts Datasheet

Client # and Name: HOW001 Howe Sound Pulp & Paper Ltd.

Sample ID: Fe Aug 12, Fe

CANTEST ID: (G# 10081333, 908130064, 908200059,

Date & Time Sampled: 2009 Aug 12@0930 , 2009 Aug 19@930,

Date & Time Received: 2009 Aug 13@ 0830 , 2009 Aug 20@957,

Date and Time	Carboy #	Volume Adjusted (L)	pH Before Salt Added	pH After Salt Added	Salinity Before Salt Added (ppt)	Salinity After Salt Added (ppt)	Mass of Salt Added (g)	Balance	Test Used For	Analyst
2009 Aug 13@1550	1	~10L	6.5	6.6	1.5	29.3	392.8	003538	Topsmelt	JR
2009 Aug 16@1530	2	~10L	6.8	6.7	1.3	29.6	372.2	003538	Topsmelt	JR
2009 Aug 18@1720	3	~10L	6.7	6.6	1.2	29.4	376.2	003538	Topsmelt	KT
2009 Aug 20@1450	4	~10L	6.6	6.4	1.3	29.4	372.1	003538	Topsmelt	JR
2009 Aug 23@1020	5	~10L	6.6	6.6	1.5	29.7	360.0	003538	Topsmelt	JR
2009 Aug 25@715	6	~10L	6.8	6.7	1.5	30.1	360.2	003538	Topsmelt	KT
2009 Aug 27@1405	7	~10L	6.6	6.7	1.0	29.2	380.0	003538	Topsmelt	KT

Description of Salt: Oceanpure Salt Lot # or Received Date: 2008 Mar 14

Comments:

Randomization Chart for Topsmelt Tests

Client Name:
Test Date:

Use the coloured tape to find appropriate concentrations and put beakers back in proper position following daily water change.

Position Map
Back Wall

5	10	15	20	25	30	35
4	9	14	19	24	29	34
3	8	13	18	23	28	33
2	7	12	17	22	27	32
1	6	11	16	21	26	31

Front of Counter

Position #	Treatment	Replicate	Colour
31	SW Control	A	Red
19	SW Control	B	Red
30	SW Control	C	Red
29	SW Control	D	Red
7	SW Control	E	Red
6	Salt Control	A	Blue
18	Salt Control	B	Blue
23	Salt Control	C	Blue
14	Salt Control	D	Blue
32	Salt Control	E	Blue
21	6.25%	A	Yellow
22	6.25%	B	Yellow
27	6.25%	C	Yellow
33	6.25%	D	Yellow
26	6.25%	E	Yellow
16	12.5%	A	Green
1	12.5%	B	Green
13	12.5%	C	Green
3	12.5%	D	Green
8	12.5%	E	Green
24	25.0%	A	Yellow Glo
17	25.0%	B	Yellow Glo
10	25.0%	C	Yellow Glo
34	25.0%	D	Yellow Glo
11	25.0%	E	Yellow Glo
2	50.0%	A	Leaf
12	50.0%	B	Leaf
5	50.0%	C	Leaf
9	50.0%	D	Leaf
35	50.0%	E	Leaf
20	100%	A	White
25	100%	B	White
4	100%	C	White
15	100%	D	White
28	100%	E	White

Orange Glo JR 2009Aug13

dark blue JR 2009Aug13

Appendix A3

AXYS Analytical: Sediment, Crab, and Finfish Analytical Reports

BATCH SUMMARY

Batch ID: WG28126	***Date:*** 20-Apr-2009
Analysis Type: Dioxin/Furan	***Matrix Type:*** Solid

BATCH MAKEUP

Contract: 2544

Samples:

L12410-1 HSDX09-SI-14

Blank: WG28126-101

Reference or Spike: WG28126-102

Duplicate: WG28126-103

Comments:

Revised on 20-Apr-2009: This data has been revised to calculate TEQ data using WHO 2005 TEF instead of WHO 1998 TEF. No other changes were made.

1. Data are not blank corrected.

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February 1993

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HSDX09-SI-14	**AXYS FILE:**	L12410-1 i
SAMPLE COLLECTION:	04-Mar-2009		
CLIENT NO.:	2544	**REPORT DATE:**	20-Apr-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	SOLID	**EXTRACTION DATE:**	10-Mar-2009
SAMPLE SIZE:	10.8 g (dry)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	26-Mar-2009
% Moisture:	56.9	**CONCENTRATION IN:**	pg/g (dry weight basis)

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	3.68	0.09
1,2,3,7,8-PECDD	5.88	0.09
1,2,3,4,7,8-HXCDD	1.34	0.19
1,2,3,6,7,8-HXCDD	96.5	0.19
1,2,3,7,8,9-HXCDD	47.7	0.19
1,2,3,4,6,7,8-HPCDD	62.2	0.19
OCDD	174	0.46
2,3,7,8-TCDF *	99.9	0.22
1,2,3,7,8-PECDF	2.18	0.09
2,3,4,7,8-PECDF	3.39	0.09
1,2,3,4,7,8-HXCDF	1.48	0.19
1,2,3,6,7,8-HXCDF	NDR (0.91)	0.19
2,3,4,6,7,8-HXCDF	1.24	0.19
1,2,3,7,8,9-HXCDF	NDR (0.23)	0.19
1,2,3,4,6,7,8-HPCDF	21.8	0.19
1,2,3,4,7,8,9-HPCDF	0.84	0.19
OCDF	16.2	0.46
TOTAL TETRA-DIOXINS	24.7	0.09
TOTAL PENTA-DIOXINS	55.3	0.09
TOTAL HEXA-DIOXINS	686	0.19
TOTAL HEPTA-DIOXINS	136	0.19
TOTAL TETRA-FURANS	220	0.09
TOTAL PENTA-FURANS	22.6	0.09
TOTAL HEXA-FURANS	34.3	0.19
TOTAL HEPTA-FURANS	48.3	0.19

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)	
2,3,7,8-TCDD TEQs (ND=0) =	36.4
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	36.4

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	53.7
13C-1,2,3,7,8-PECDD	52.9
13C-1,2,3,6,7,8-HXCDD	54.0
13C-1,2,3,4,6,7,8-HPCDD	56.1
13C-OCDD	49.0
13C-2,3,7,8-TCDF	52.8
13C-1,2,3,7,8-PECDF	53.0
13C-1,2,3,4,7,8-HXCDF	54.4
13C-1,2,3,4,6,7,8-HPCDF	53.5

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_L12410-1_SJ993795.html; Workgroup: WG28126; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	Lab Blank	**AXYS FILE:**	WG28126-101
SAMPLE COLLECTION:	N/A		
CLIENT NO.:	2544	**REPORT DATE:**	20-Apr-2009
PROJECT NO.:	N/A	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	SOLID	**EXTRACTION DATE:**	10-Mar-2009
SAMPLE SIZE:	10.0 g	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	16-Mar-2009
		CONCENTRATION IN:	pg/g

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	ND	0.10
1,2,3,7,8-PECDD	ND	0.10
1,2,3,4,7,8-HXCDD	ND	0.20
1,2,3,6,7,8-HXCDD	ND	0.20
1,2,3,7,8,9-HXCDD	ND	0.20
1,2,3,4,6,7,8-HPCDD	ND	0.20
OCDD	ND	0.50
2,3,7,8-TCDF	ND	0.10
1,2,3,7,8-PECDF	ND	0.10
2,3,4,7,8-PECDF	ND	0.10
1,2,3,4,7,8-HXCDF	ND	0.20
1,2,3,6,7,8-HXCDF	ND	0.20
2,3,4,6,7,8-HXCDF	ND	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	ND	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.50
TOTAL TETRA-DIOXINS	ND	0.10
TOTAL PENTA-DIOXINS	ND	0.10
TOTAL HEXA-DIOXINS	ND	0.20
TOTAL HEPTA-DIOXINS	ND	0.20
TOTAL TETRA-FURANS	ND	0.10
TOTAL PENTA-FURANS	ND	0.10
TOTAL HEXA-FURANS	ND	0.20
TOTAL HEPTA-FURANS	ND	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)	
2,3,7,8-TCDD TEQs (ND=0) =	0
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	0.19

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	54.9
13C-1,2,3,7,8-PECDD	60.2
13C-1,2,3,6,7,8-HXCDD	57.1
13C-1,2,3,4,6,7,8-HPCDD	62.7
13C-OCDD	56.2
13C-2,3,7,8-TCDF	53.6
13C-1,2,3,7,8-PECDF	59.3
13C-1,2,3,4,7,8-HXCDF	60.1
13C-1,2,3,4,6,7,8-HPCDF	59.2

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

For Axys Internal Use Only [XSL Template: AXYS.xsl; Created: 20-Apr-2009 14:39:12; Application: XMLTransformer-1.9.22;
Report Filename: AXYS_DIOXINS_AXYSDB5_WG28126-101_SJ991427.html; Workgroup: WG28126; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	Spiked Matrix	**AXYS FILE:**	WG28126-102
CLIENT NO.:	2544	**REPORT DATE:**	20-Apr-2009
SAMPLE TYPE:	SOLID	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE SIZE:	10.0 g	**INSTRUMENT:**	HR GC/MS
		CONCENTRATION IN:	pg/g

COMPOUND	Determined	Expected	% Recovery
2,3,7,8-TCDD	10.0	10.6	94.5
1,2,3,7,8-PECDD	52.1	56.6	92.0
1,2,3,4,7,8-HXCDD	55.7	59.2	94.1
1,2,3,6,7,8-HXCDD	54.7	51.8	106
1,2,3,7,8,9-HXCDD	56.9	56.7	100
1,2,3,4,6,7,8-HPCDD	45.6	50.0	91.2
OCDD	97.0	108	89.9
2,3,7,8-TCDF	10.4	10.9	95.7
1,2,3,7,8-PECDF	41.7	50.0	83.5
2,3,4,7,8-PECDF	42.1	50.0	84.1
1,2,3,4,7,8-HXCDF	48.0	54.4	88.3
1,2,3,6,7,8-HXCDF	44.8	50.0	89.6
2,3,4,6,7,8-HXCDF	50.8	53.1	95.6
1,2,3,7,8,9-HXCDF	48.5	50.0	97.1
1,2,3,4,6,7,8-HPCDF	51.8	50.0	104
1,2,3,4,7,8,9-HPCDF	49.6	50.0	99.3
OCDF	96.5	109	88.8

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	58.7
13C-1,2,3,7,8-PECDD	56.9
13C-1,2,3,6,7,8-HXCDD	59.2
13C-1,2,3,4,6,7,8-HPCDD	64.6
13C-OCDD	59.9
13C-2,3,7,8-TCDF	59.5
13C-1,2,3,7,8-PECDF	62.4
13C-1,2,3,4,7,8-HXCDF	62.8
13C-1,2,3,4,6,7,8-HPCDF	61.9

1. Concentrations are recovery corrected

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_WG28126-102_SJ991426.html; Workgroup: WG28126; Design ID: 83]

BATCH SUMMARY

Batch ID: WG28522	***Date:*** 25-May-2009
Analysis Type: Dioxin/Furan	***Matrix Type:*** Tissue

BATCH MAKEUP

Contract: 2544

Samples:

L12549-1	HS1489-C9
L12549-2	HS1489-C10
L12549-3	HS1489-C14
L12549-4	HS1489-C16a
L12549-5	HS1489-C19a
L12549-6	HS1489-C21
L12549-7	HS1489-C27

Blank: WG28522-101

Reference or Spike: WG28522-102

Duplicate: WG28522-103

Comments:

1. Data are not blank corrected.

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C9	**AXYS FILE:**	L12549-1 (A)
SAMPLE COLLECTION:	06-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	10.2 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	30-Apr-2009
% Moisture:	83.9	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	7.26		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	3.17	0.10
1,2,3,7,8-PECDD	14.9	0.10
1,2,3,4,7,8-HXCDD	2.74	0.20
1,2,3,6,7,8-HXCDD	112	0.20
1,2,3,7,8,9-HXCDD	14.7	0.20
1,2,3,4,6,7,8-HPCDD	3.79	0.20
OCDD	1.49	0.49
2,3,7,8-TCDF *	103	0.10
1,2,3,7,8-PECDF	0.83	0.10
2,3,4,7,8-PECDF	6.39	0.10
1,2,3,4,7,8-HXCDF	1.70	0.20
1,2,3,6,7,8-HXCDF	NDR (0.30)	0.20
2,3,4,6,7,8-HXCDF	1.35	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	2.78	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.49
TOTAL TETRA-DIOXINS	11.3	0.10
TOTAL PENTA-DIOXINS	72.1	0.10
TOTAL HEXA-DIOXINS	319	0.20
TOTAL HEPTA-DIOXINS	7.46	0.20
TOTAL TETRA-FURANS	177	0.10
TOTAL PENTA-FURANS	44.7	0.10
TOTAL HEXA-FURANS	26.7	0.20
TOTAL HEPTA-FURANS	2.78	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	43.6
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	43.6

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	88.0
13C-1,2,3,7,8-PECDD	102
13C-1,2,3,6,7,8-HXCDD	78.5
13C-1,2,3,4,6,7,8-HPCDD	94.8
13C-OCDD	101
13C-2,3,7,8-TCDF	88.0
13C-1,2,3,7,8-PECDF	93.6
13C-1,2,3,4,7,8-HXCDF	78.4
13C-1,2,3,4,6,7,8-HPCDF	86.3

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_L12549-1_SJ1007763.html; Workgroup: WG28522; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C10	**AXYS FILE:**	L12549-2
SAMPLE COLLECTION:	07-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	10.3 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	30-Apr-2009
% Moisture:	84.6	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	7.58		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	2.66	0.10
1,2,3,7,8-PECDD	11.3	0.10
1,2,3,4,7,8-HXCDD	1.10	0.20
1,2,3,6,7,8-HXCDD	87.9	0.20
1,2,3,7,8,9-HXCDD	18.5	0.20
1,2,3,4,6,7,8-HPCDD	5.86	0.20
OCDD	1.88	0.49
2,3,7,8-TCDF *	88.7	0.10
1,2,3,7,8-PECDF	1.04	0.10
2,3,4,7,8-PECDF	3.60	0.10
1,2,3,4,7,8-HXCDF	0.67	0.20
1,2,3,6,7,8-HXCDF	0.33	0.20
2,3,4,6,7,8-HXCDF	0.62	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	1.64	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.49
TOTAL TETRA-DIOXINS	6.74	0.10
TOTAL PENTA-DIOXINS	43.4	0.10
TOTAL HEXA-DIOXINS	335	0.20
TOTAL HEPTA-DIOXINS	11.1	0.20
TOTAL TETRA-FURANS	141	0.10
TOTAL PENTA-FURANS	24.5	0.10
TOTAL HEXA-FURANS	10.7	0.20
TOTAL HEPTA-FURANS	1.64	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	34.9
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	34.9

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	61.9
13C-1,2,3,7,8-PECDD	65.5
13C-1,2,3,6,7,8-HXCDD	65.0
13C-1,2,3,4,6,7,8-HPCDD	62.1
13C-OCDD	63.7
13C-2,3,7,8-TCDF	59.9
13C-1,2,3,7,8-PECDF	58.0
13C-1,2,3,4,7,8-HXCDF	62.0
13C-1,2,3,4,6,7,8-HPCDF	62.7

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C14	**AXYS FILE:**	L12549-3
SAMPLE COLLECTION:	06-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	10.8 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	30-Apr-2009
% Moisture:	84.6	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	5.62		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	2.22	0.09
1,2,3,7,8-PECDD	7.48	0.09
1,2,3,4,7,8-HXCDD	1.02	0.18
1,2,3,6,7,8-HXCDD	40.4	0.18
1,2,3,7,8,9-HXCDD	7.50	0.18
1,2,3,4,6,7,8-HPCDD	4.72	0.18
OCDD	3.87	0.46
2,3,7,8-TCDF *	80.9	0.09
1,2,3,7,8-PECDF	0.87	0.09
2,3,4,7,8-PECDF	3.27	0.09
1,2,3,4,7,8-HXCDF	0.67	0.18
1,2,3,6,7,8-HXCDF	0.34	0.18
2,3,4,6,7,8-HXCDF	0.51	0.18
1,2,3,7,8,9-HXCDF	ND	0.18
1,2,3,4,6,7,8-HPCDF	1.91	0.18
1,2,3,4,7,8,9-HPCDF	ND	0.18
OCDF	ND	0.46
TOTAL TETRA-DIOXINS	9.17	0.09
TOTAL PENTA-DIOXINS	32.1	0.09
TOTAL HEXA-DIOXINS	132	0.18
TOTAL HEPTA-DIOXINS	10.8	0.18
TOTAL TETRA-FURANS	135	0.09
TOTAL PENTA-FURANS	20.3	0.09
TOTAL HEXA-FURANS	10.4	0.18
TOTAL HEPTA-FURANS	2.51	0.18

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	23.9
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	23.9

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	78.6
13C-1,2,3,7,8-PECDD	87.8
13C-1,2,3,6,7,8-HXCDD	81.5
13C-1,2,3,4,6,7,8-HPCDD	77.0
13C-OCDD	73.5
13C-2,3,7,8-TCDF	75.6
13C-1,2,3,7,8-PECDF	77.8
13C-1,2,3,4,7,8-HXCDF	79.2
13C-1,2,3,4,6,7,8-HPCDF	79.6

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C16a	**AXYS FILE:**	L12549-4
SAMPLE COLLECTION:	07-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	10.2 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	30-Apr-2009
% Moisture:	82.5	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	7.91		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	1.94	0.10
1,2,3,7,8-PECDD	7.99	0.10
1,2,3,4,7,8-HXCDD	1.42	0.20
1,2,3,6,7,8-HXCDD	22.1	0.20
1,2,3,7,8,9-HXCDD	5.33	0.20
1,2,3,4,6,7,8-HPCDD	7.69	0.20
OCDD	2.89	0.49
2,3,7,8-TCDF *	45.7	0.10
1,2,3,7,8-PECDF	1.04	0.10
2,3,4,7,8-PECDF	2.39	0.10
1,2,3,4,7,8-HXCDF	NDR (0.28)	0.20
1,2,3,6,7,8-HXCDF	0.62	0.20
2,3,4,6,7,8-HXCDF	0.59	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	2.59	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.49
TOTAL TETRA-DIOXINS	8.50	0.10
TOTAL PENTA-DIOXINS	29.9	0.10
TOTAL HEXA-DIOXINS	84.0	0.20
TOTAL HEPTA-DIOXINS	15.4	0.20
TOTAL TETRA-FURANS	79.1	0.10
TOTAL PENTA-FURANS	20.7	0.10
TOTAL HEXA-FURANS	12.0	0.20
TOTAL HEPTA-FURANS	2.92	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	18.4
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	18.4

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	66.2
13C-1,2,3,7,8-PECDD	72.6
13C-1,2,3,6,7,8-HXCDD	72.0
13C-1,2,3,4,6,7,8-HPCDD	67.6
13C-OCDD	62.9
13C-2,3,7,8-TCDF	64.5
13C-1,2,3,7,8-PECDF	66.9
13C-1,2,3,4,7,8-HXCDF	69.8
13C-1,2,3,4,6,7,8-HPCDF	69.1

1. SDL = Sample Detection Limit
2. ND = Not detected
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4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C19a	**AXYS FILE:**	L12549-5
SAMPLE COLLECTION:	07-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	2.85 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	30-Apr-2009
% Moisture:	82.3	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	7.74		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	5.61	0.35
1,2,3,7,8-PECDD	30.3	0.35
1,2,3,4,7,8-HXCDD	6.33	0.70
1,2,3,6,7,8-HXCDD	180	0.70
1,2,3,7,8,9-HXCDD	23.3	0.70
1,2,3,4,6,7,8-HPCDD	13.5	0.70
OCDD	7.43	1.75
2,3,7,8-TCDF *	233	0.35
1,2,3,7,8-PECDF	2.08	0.35
2,3,4,7,8-PECDF	15.0	0.35
1,2,3,4,7,8-HXCDF	4.59	0.70
1,2,3,6,7,8-HXCDF	1.01	0.70
2,3,4,6,7,8-HXCDF	4.91	0.70
1,2,3,7,8,9-HXCDF	ND	0.70
1,2,3,4,6,7,8-HPCDF	11.1	0.70
1,2,3,4,7,8,9-HPCDF	ND	0.70
OCDF	ND	1.75
TOTAL TETRA-DIOXINS	20.2	0.35
TOTAL PENTA-DIOXINS	121	0.35
TOTAL HEXA-DIOXINS	489	0.70
TOTAL HEPTA-DIOXINS	24.9	0.70
TOTAL TETRA-FURANS	370	0.35
TOTAL PENTA-FURANS	111	0.35
TOTAL HEXA-FURANS	98.5	0.70
TOTAL HEPTA-FURANS	12.4	0.70

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	86.0
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	86.1

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	71.2
13C-1,2,3,7,8-PECDD	71.4
13C-1,2,3,6,7,8-HXCDD	79.8
13C-1,2,3,4,6,7,8-HPCDD	74.6
13C-OCDD	69.6
13C-2,3,7,8-TCDF	70.4
13C-1,2,3,7,8-PECDF	69.6
13C-1,2,3,4,7,8-HXCDF	75.0
13C-1,2,3,4,6,7,8-HPCDF	75.9

1. SDL = Sample Detection Limit
2. ND = Not detected
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4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C21	**AXYS FILE:**	L12549-6
SAMPLE COLLECTION:	06-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	10.2 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	01-May-2009
% Moisture:	72.4	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	15.9		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	0.93	0.10
1,2,3,7,8-PECDD	3.31	0.10
1,2,3,4,7,8-HXCDD	0.91	0.20
1,2,3,6,7,8-HXCDD	19.4	0.20
1,2,3,7,8,9-HXCDD	4.97	0.20
1,2,3,4,6,7,8-HPCDD	7.51	0.20
OCDD	4.39	0.49
2,3,7,8-TCDF *	26.2	0.10
1,2,3,7,8-PECDF	0.55	0.10
2,3,4,7,8-PECDF	1.76	0.10
1,2,3,4,7,8-HXCDF	0.74	0.20
1,2,3,6,7,8-HXCDF	0.41	0.20
2,3,4,6,7,8-HXCDF	0.65	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	3.63	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	0.58	0.49
TOTAL TETRA-DIOXINS	6.59	0.10
TOTAL PENTA-DIOXINS	15.9	0.10
TOTAL HEXA-DIOXINS	80.7	0.20
TOTAL HEPTA-DIOXINS	14.3	0.20
TOTAL TETRA-FURANS	51.0	0.10
TOTAL PENTA-FURANS	16.9	0.10
TOTAL HEXA-FURANS	15.8	0.20
TOTAL HEPTA-FURANS	5.58	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	10.2
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	10.2

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	60.9
13C-1,2,3,7,8-PECDD	61.6
13C-1,2,3,6,7,8-HXCDD	69.1
13C-1,2,3,4,6,7,8-HPCDD	63.8
13C-OCDD	51.9
13C-2,3,7,8-TCDF	59.9
13C-1,2,3,7,8-PECDF	60.3
13C-1,2,3,4,7,8-HXCDF	66.7
13C-1,2,3,4,6,7,8-HPCDF	65.2

1. SDL = Sample Detection Limit
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4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C27	**AXYS FILE:**	L12549-7
SAMPLE COLLECTION:	07-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	5.45 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	01-May-2009
% Moisture:	84.3	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	6.95		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	1.43	0.18
1,2,3,7,8-PECDD	5.89	0.18
1,2,3,4,7,8-HXCDD	0.76	0.37
1,2,3,6,7,8-HXCDD	26.0	0.37
1,2,3,7,8,9-HXCDD	5.44	0.37
1,2,3,4,6,7,8-HPCDD	2.55	0.37
OCDD	ND	0.92
2,3,7,8-TCDF *	37.6	0.18
1,2,3,7,8-PECDF	0.76	0.18
2,3,4,7,8-PECDF	1.96	0.18
1,2,3,4,7,8-HXCDF	0.59	0.37
1,2,3,6,7,8-HXCDF	NDR (0.37)	0.37
2,3,4,6,7,8-HXCDF	0.49	0.37
1,2,3,7,8,9-HXCDF	ND	0.37
1,2,3,4,6,7,8-HPCDF	1.52	0.37
1,2,3,4,7,8,9-HPCDF	ND	0.37
OCDF	ND	0.92
TOTAL TETRA-DIOXINS	5.21	0.18
TOTAL PENTA-DIOXINS	17.4	0.18
TOTAL HEXA-DIOXINS	82.0	0.37
TOTAL HEPTA-DIOXINS	2.55	0.37
TOTAL TETRA-FURANS	64.2	0.18
TOTAL PENTA-FURANS	12.7	0.18
TOTAL HEXA-FURANS	7.82	0.37
TOTAL HEPTA-FURANS	1.52	0.37

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	15.1
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	15.1

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	72.3
13C-1,2,3,7,8-PECDD	77.9
13C-1,2,3,6,7,8-HXCDD	84.7
13C-1,2,3,4,6,7,8-HPCDD	75.9
13C-OCDD	66.5
13C-2,3,7,8-TCDF	71.2
13C-1,2,3,7,8-PECDF	70.5
13C-1,2,3,4,7,8-HXCDF	80.4
13C-1,2,3,4,6,7,8-HPCDF	80.8

1. SDL = Sample Detection Limit
2. ND = Not detected
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4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	Lab Blank	**AXYS FILE:**	WG28522-101
SAMPLE COLLECTION:	N/A		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	N/A	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	10.0 g	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	30-Apr-2009
CONCENTRATION IN:	pg/g		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	ND	0.10
1,2,3,7,8-PECDD	ND	0.10
1,2,3,4,7,8-HXCDD	ND	0.20
1,2,3,6,7,8-HXCDD	ND	0.20
1,2,3,7,8,9-HXCDD	ND	0.20
1,2,3,4,6,7,8-HPCDD	ND	0.20
OCDD	ND	0.50
2,3,7,8-TCDF	ND	0.10
1,2,3,7,8-PECDF	ND	0.10
2,3,4,7,8-PECDF	ND	0.10
1,2,3,4,7,8-HXCDF	ND	0.20
1,2,3,6,7,8-HXCDF	ND	0.20
2,3,4,6,7,8-HXCDF	ND	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	ND	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.50
TOTAL TETRA-DIOXINS	ND	0.10
TOTAL PENTA-DIOXINS	ND	0.10
TOTAL HEXA-DIOXINS	ND	0.20
TOTAL HEPTA-DIOXINS	ND	0.20
TOTAL TETRA-FURANS	ND	0.10
TOTAL PENTA-FURANS	ND	0.10
TOTAL HEXA-FURANS	ND	0.20
TOTAL HEPTA-FURANS	ND	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)	
2,3,7,8-TCDD TEQs (ND=0) =	0
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	0.19

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	78.9
13C-1,2,3,7,8-PECDD	84.8
13C-1,2,3,6,7,8-HXCDD	75.2
13C-1,2,3,4,6,7,8-HPCDD	76.3
13C-OCDD	67.2
13C-2,3,7,8-TCDF	76.9
13C-1,2,3,7,8-PECDF	76.1
13C-1,2,3,4,7,8-HXCDF	79.9
13C-1,2,3,4,6,7,8-HPCDF	80.2

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	Spiked Matrix	**AXYS FILE:**	WG28522-102
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
SAMPLE TYPE:	TISSUE	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE SIZE:	10.0 g	**INSTRUMENT:**	HR GC/MS
		CONCENTRATION IN:	pg/g

COMPOUND	Determined	Expected	% Recovery
2,3,7,8-TCDD	10.8	10.6	102
1,2,3,7,8-PECDD	53.8	56.6	95.1
1,2,3,4,7,8-HXCDD	56.3	59.2	95.1
1,2,3,6,7,8-HXCDD	55.9	51.8	108
1,2,3,7,8,9-HXCDD	56.0	56.7	98.7
1,2,3,4,6,7,8-HPCDD	48.2	50.0	96.5
OCDD	101	108	93.5
2,3,7,8-TCDF	10.7	10.9	98.2
1,2,3,7,8-PECDF	44.8	50.0	89.5
2,3,4,7,8-PECDF	45.7	50.0	91.4
1,2,3,4,7,8-HXCDF	48.3	54.4	88.9
1,2,3,6,7,8-HXCDF	48.5	50.0	97.0
2,3,4,6,7,8-HXCDF	54.7	53.1	103
1,2,3,7,8,9-HXCDF	58.9	50.0	118
1,2,3,4,6,7,8-HPCDF	51.2	50.0	102
1,2,3,4,7,8,9-HPCDF	49.8	50.0	99.6
OCDF	105	109	97.1

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	64.1
13C-1,2,3,7,8-PECDD	68.8
13C-1,2,3,6,7,8-HXCDD	64.5
13C-1,2,3,4,6,7,8-HPCDD	63.9
13C-OCDD	64.4
13C-2,3,7,8-TCDF	61.5
13C-1,2,3,7,8-PECDF	59.9
13C-1,2,3,4,7,8-HXCDF	60.0
13C-1,2,3,4,6,7,8-HPCDF	63.9

1. Concentrations are recovery corrected

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_WG28522-102_SJ1007761.html; Workgroup: WG28522; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HS1489-C9 (Duplicate)	**AXYS FILE:**	WG28522-103 (DUP L12549-1)
SAMPLE COLLECTION:	06-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	25-May-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	17-Apr-2009
SAMPLE SIZE:	10.5 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	30-Apr-2009
% Moisture:	84.0	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	6.84		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	3.11	0.10
1,2,3,7,8-PECDD	15.7	0.10
1,2,3,4,7,8-HXCDD	2.16	0.19
1,2,3,6,7,8-HXCDD	112	0.19
1,2,3,7,8,9-HXCDD	13.4	0.19
1,2,3,4,6,7,8-HPCDD	4.23	0.19
OCDD	1.40	0.48
2,3,7,8-TCDF *	101	0.10
1,2,3,7,8-PECDF	0.94	0.10
2,3,4,7,8-PECDF	6.44	0.10
1,2,3,4,7,8-HXCDF	1.65	0.19
1,2,3,6,7,8-HXCDF	0.32	0.19
2,3,4,6,7,8-HXCDF	1.29	0.19
1,2,3,7,8,9-HXCDF	ND	0.19
1,2,3,4,6,7,8-HPCDF	2.85	0.19
1,2,3,4,7,8,9-HPCDF	ND	0.19
OCDF	ND	0.48
TOTAL TETRA-DIOXINS	11.2	0.10
TOTAL PENTA-DIOXINS	73.5	0.10
TOTAL HEXA-DIOXINS	320	0.19
TOTAL HEPTA-DIOXINS	8.51	0.19
TOTAL TETRA-FURANS	186	0.10
TOTAL PENTA-FURANS	47.7	0.10
TOTAL HEXA-FURANS	28.6	0.19
TOTAL HEPTA-FURANS	2.85	0.19

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	44.0
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	44.0

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	73.8
13C-1,2,3,7,8-PECDD	82.9
13C-1,2,3,6,7,8-HXCDD	79.5
13C-1,2,3,4,6,7,8-HPCDD	77.6
13C-OCDD	72.9
13C-2,3,7,8-TCDF	72.1
13C-1,2,3,7,8-PECDF	74.3
13C-1,2,3,4,7,8-HXCDF	79.5
13C-1,2,3,4,6,7,8-HPCDF	81.0

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Shelley Facchin_____ QA/QC Chemist

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BATCH SUMMARY

Batch ID: WG28670	***Date:*** 02-Jun-2009
Analysis Type: Dioxin/Furan	***Matrix Type:*** Tissue

BATCH MAKEUP

Contract: 2544

Samples:

L12618-1	HSDX09-T9-DF-L
L12618-2	HSDX09-T9-DF-M
L12618-3	HSDX09-T9-SL-L

Blank: WG28670-101

Reference or Spike: WG28670-102

Duplicate:

Comments:

1. Data are not blank corrected.

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ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HSDX09-T9-DF-L	**AXYS FILE:**	L12618-1
SAMPLE COLLECTION:	28-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	02-Jun-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	04-May-2009
SAMPLE SIZE:	9.98 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	08-May-2009
% Moisture:	30.9	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	65.4		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	218	0.10
1,2,3,7,8-PECDD	247	0.10
1,2,3,4,7,8-HXCDD	5.49	0.20
1,2,3,6,7,8-HXCDD	508	0.20
1,2,3,7,8,9-HXCDD	45.8	0.20
1,2,3,4,6,7,8-HPCDD	7.93	0.20
OCDD	1.76	0.50
2,3,7,8-TCDF *	4900	0.10
1,2,3,7,8-PECDF	22.4	0.10
2,3,4,7,8-PECDF	95.8	0.10
1,2,3,4,7,8-HXCDF	2.53	0.20
1,2,3,6,7,8-HXCDF	2.66	0.20
2,3,4,6,7,8-HXCDF	2.70	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	1.17	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.50
TOTAL TETRA-DIOXINS	220	0.10
TOTAL PENTA-DIOXINS	249	0.10
TOTAL HEXA-DIOXINS	563	0.20
TOTAL HEPTA-DIOXINS	7.93	0.20
TOTAL TETRA-FURANS	4530	0.10
TOTAL PENTA-FURANS	124	0.10
TOTAL HEXA-FURANS	10.5	0.20
TOTAL HEPTA-FURANS	1.45	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	1040
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	1040

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	69.6
13C-1,2,3,7,8-PECDD	73.8
13C-1,2,3,6,7,8-HXCDD	71.5
13C-1,2,3,4,6,7,8-HPCDD	63.6
13C-OCDD	61.4
13C-2,3,7,8-TCDF	76.3
13C-1,2,3,7,8-PECDF	69.8
13C-1,2,3,4,7,8-HXCDF	65.1
13C-1,2,3,4,6,7,8-HPCDF	63.9

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Teresa Rawsthorne_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_L12618-1_SJ1013531.html; Workgroup: WG28670; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HSDX09-T9-DF-M	**AXYS FILE:**	L12618-2
SAMPLE COLLECTION:	28-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	02-Jun-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	04-May-2009
SAMPLE SIZE:	10.2 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	08-May-2009
% Moisture:	70.6	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	67.5		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	2.03	0.10
1,2,3,7,8-PECDD	1.75	0.10
1,2,3,4,7,8-HXCDD	ND	0.20
1,2,3,6,7,8-HXCDD	7.62	0.20
1,2,3,7,8,9-HXCDD	0.69	0.20
1,2,3,4,6,7,8-HPCDD	0.39	0.20
OCDD	ND	0.49
2,3,7,8-TCDF *	19.2	0.10
1,2,3,7,8-PECDF	NDR (0.17)	0.10
2,3,4,7,8-PECDF	0.68	0.10
1,2,3,4,7,8-HXCDF	ND	0.20
1,2,3,6,7,8-HXCDF	ND	0.20
2,3,4,6,7,8-HXCDF	ND	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	ND	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.49
TOTAL TETRA-DIOXINS	2.03	0.10
TOTAL PENTA-DIOXINS	1.75	0.10
TOTAL HEXA-DIOXINS	8.31	0.20
TOTAL HEPTA-DIOXINS	0.39	0.20
TOTAL TETRA-FURANS	17.5	0.10
TOTAL PENTA-FURANS	0.68	0.10
TOTAL HEXA-FURANS	ND	0.20
TOTAL HEPTA-FURANS	ND	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	6.74
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	6.79

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	61.5
13C-1,2,3,7,8-PECDD	64.8
13C-1,2,3,6,7,8-HXCDD	70.9
13C-1,2,3,4,6,7,8-HPCDD	59.3
13C-OCDD	52.1
13C-2,3,7,8-TCDF	63.3
13C-1,2,3,7,8-PECDF	62.5
13C-1,2,3,4,7,8-HXCDF	65.3
13C-1,2,3,4,6,7,8-HPCDF	61.3

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Teresa Rawsthorne_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_L12618-2_SJ1013519.html; Workgroup: WG28670; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	HSDX09-T9-SL-L	**AXYS FILE:**	L12618-3
SAMPLE COLLECTION:	28-Apr-2009		
CLIENT NO.:	2544	**REPORT DATE:**	02-Jun-2009
PROJECT NO.:	HS1489	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	TISSUE	**EXTRACTION DATE:**	04-May-2009
SAMPLE SIZE:	3.97 g (wet)	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	08-May-2009
% Moisture:	42.5	**CONCENTRATION IN:**	pg/g (wet weight basis)
% Lipid:	49.0		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	2.30	0.25
1,2,3,7,8-PECDD	5.03	0.25
1,2,3,4,7,8-HXCDD	ND	0.50
1,2,3,6,7,8-HXCDD	8.37	0.50
1,2,3,7,8,9-HXCDD	0.76	0.50
1,2,3,4,6,7,8-HPCDD	ND	0.50
OCDD	ND	1.26
2,3,7,8-TCDF *	48.6	0.25
1,2,3,7,8-PECDF	0.59	0.25
2,3,4,7,8-PECDF	2.43	0.25
1,2,3,4,7,8-HXCDF	ND	0.50
1,2,3,6,7,8-HXCDF	ND	0.50
2,3,4,6,7,8-HXCDF	ND	0.50
1,2,3,7,8,9-HXCDF	ND	0.50
1,2,3,4,6,7,8-HPCDF	ND	0.50
1,2,3,4,7,8,9-HPCDF	ND	0.50
OCDF	ND	1.26
TOTAL TETRA-DIOXINS	2.30	0.25
TOTAL PENTA-DIOXINS	5.03	0.25
TOTAL HEXA-DIOXINS	9.13	0.50
TOTAL HEPTA-DIOXINS	ND	0.50
TOTAL TETRA-FURANS	44.4	0.25
TOTAL PENTA-FURANS	3.95	0.25
TOTAL HEXA-FURANS	0.74	0.50
TOTAL HEPTA-FURANS	ND	0.50

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)

2,3,7,8-TCDD TEQs (ND=0) =	13.8
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	14.0

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	72.2
13C-1,2,3,7,8-PECDD	76.0
13C-1,2,3,6,7,8-HXCDD	81.0
13C-1,2,3,4,6,7,8-HPCDD	69.9
13C-OCDD	64.2
13C-2,3,7,8-TCDF	71.5
13C-1,2,3,7,8-PECDF	72.0
13C-1,2,3,4,7,8-HXCDF	74.7
13C-1,2,3,4,6,7,8-HPCDF	72.3

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Teresa Rawsthorne_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_L12618-3_SJ1013520.html; Workgroup: WG28670; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	Lab Blank	**AXYS FILE:**	WG28670-101 i
SAMPLE COLLECTION:	N/A		
CLIENT NO.:	2544	**REPORT DATE:**	02-Jun-2009
PROJECT NO.:	N/A	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE TYPE:	CORN OIL	**EXTRACTION DATE:**	04-May-2009
SAMPLE SIZE:	10.0 g	**INSTRUMENT:**	HR GC/MS
		ANALYSIS DATE:	07-May-2009
CONCENTRATION IN:	pg/g		

COMPOUND	Concentration	(SDL)
2,3,7,8-TCDD	ND	0.10
1,2,3,7,8-PECDD	ND	0.10
1,2,3,4,7,8-HXCDD	ND	0.20
1,2,3,6,7,8-HXCDD	ND	0.20
1,2,3,7,8,9-HXCDD	ND	0.20
1,2,3,4,6,7,8-HPCDD	ND	0.20
OCDD	ND	0.50
2,3,7,8-TCDF *	ND	0.10
1,2,3,7,8-PECDF	ND	0.10
2,3,4,7,8-PECDF	ND	0.10
1,2,3,4,7,8-HXCDF	ND	0.20
1,2,3,6,7,8-HXCDF	ND	0.20
2,3,4,6,7,8-HXCDF	ND	0.20
1,2,3,7,8,9-HXCDF	ND	0.20
1,2,3,4,6,7,8-HPCDF	ND	0.20
1,2,3,4,7,8,9-HPCDF	ND	0.20
OCDF	ND	0.50
TOTAL TETRA-DIOXINS	ND	0.10
TOTAL PENTA-DIOXINS	ND	0.10
TOTAL HEXA-DIOXINS	ND	0.20
TOTAL HEPTA-DIOXINS	ND	0.20
TOTAL TETRA-FURANS	ND	0.10
TOTAL PENTA-FURANS	ND	0.10
TOTAL HEXA-FURANS	ND	0.20
TOTAL HEPTA-FURANS	ND	0.20

2,3,7,8-TCDD TEQs (Using WHO 2005 TEFs)	
2,3,7,8-TCDD TEQs (ND=0) =	0
2,3,7,8-TCDD TEQs (ND=1/2 DL) =	0.19

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	72.7
13C-1,2,3,7,8-PECDD	79.8
13C-1,2,3,6,7,8-HXCDD	75.2
13C-1,2,3,4,6,7,8-HPCDD	64.4
13C-OCDD	58.5
13C-2,3,7,8-TCDF	71.0
13C-1,2,3,7,8-PECDF	76.0
13C-1,2,3,4,7,8-HXCDF	68.1
13C-1,2,3,4,6,7,8-HPCDF	67.1

1. SDL = Sample Detection Limit
2. ND = Not detected
3. NDR = peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration
4. Concentrations are recovery corrected
5. * = Concentration confirmed by analysis with DB-225 column

Approved by: _____Teresa Rawsthorne_____ QA/QC Chemist

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Report Filename: AXYS_DIOXINS_AXYSDB5_WG28670-101_SJ1013539.html; Workgroup: WG28670; Design ID: 83]

ANALYSIS REPORT
POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

AXYS ANALYTICAL SERVICES
2045 MILLS RD., SIDNEY, B.C., CANADA
V8L 5X2 TEL (250) 655-5800 FAX (250) 655-5811

CLIENT SAMPLE I.D.:	Spiked Matrix	**AXYS FILE:**	WG28670-102 i
CLIENT NO.:	2544	**REPORT DATE:**	02-Jun-2009
SAMPLE TYPE:	TISSUE	**METHOD NO.:**	AXYS METHOD MLA-017 Rev 16
SAMPLE SIZE:	10.0 g	**INSTRUMENT:**	HR GC/MS
		CONCENTRATION IN:	pg/g

COMPOUND	Determined	Expected	% Recovery
2,3,7,8-TCDD	10.5	10.6	99.5
1,2,3,7,8-PECDD	52.4	56.6	92.6
1,2,3,4,7,8-HXCDD	52.4	59.2	88.4
1,2,3,6,7,8-HXCDD	52.2	51.8	101
1,2,3,7,8,9-HXCDD	48.5	56.7	85.5
1,2,3,4,6,7,8-HPCDD	46.1	50.0	92.1
OCDD	97.2	108	90.1
2,3,7,8-TCDF	10.6	10.9	96.8
1,2,3,7,8-PECDF	42.9	50.0	85.8
2,3,4,7,8-PECDF	46.0	50.0	92.0
1,2,3,4,7,8-HXCDF	46.5	54.4	85.5
1,2,3,6,7,8-HXCDF	47.5	50.0	95.0
2,3,4,6,7,8-HXCDF	54.5	53.1	103
1,2,3,7,8,9-HXCDF	57.8	50.0	116
1,2,3,4,6,7,8-HPCDF	50.2	50.0	100
1,2,3,4,7,8,9-HPCDF	50.0	50.0	100
OCDF	103	109	95.1

Surrogate Standards	% Recovery
13C-2,3,7,8-TCDD	66.4
13C-1,2,3,7,8-PECDD	70.8
13C-1,2,3,6,7,8-HXCDD	71.6
13C-1,2,3,4,6,7,8-HPCDD	60.9
13C-OCDD	54.2
13C-2,3,7,8-TCDF	65.6
13C-1,2,3,7,8-PECDF	68.8
13C-1,2,3,4,7,8-HXCDF	62.7
13C-1,2,3,4,6,7,8-HPCDF	60.7

1. Concentrations are recovery corrected

Approved by: _____Teresa Rawsthorne_____ QA/QC Chemist

For Axys Internal Use Only [XSL Template: AXYSSpike.xsl; Created: 02-Jun-2009 09:06:55; Application: XMLTransformer-1.9.24;
Report Filename: AXYS_DIOXINS_AXYSDB5_WG28670-102_SJ1013536.html; Workgroup: WG28670; Design ID: 83]

Appendix A4

ALS Environmental:
Sediment Analytical Reports

Certificate of Analysis

HATFIELD CONSULTANTS LTD.
ATTN: NARA HENDERSON
201 - 1571 BELLEVUE AVE.
WEST VANCOUVER BC V7V 1A6

Reported On: 12-MAR-09 04:00 PM
Revision: 1

Lab Work Order #: **L739693** **Date Received:** **05-MAR-09**

Project P.O. #: HOWE SOUND
Job Reference: HS1489
Legal Site Desc:
CofC Numbers:

Other Information:

Comments:

Natasha Markovic-M.

NATASHA MARKOVIC-MIROVIC
Account Manager

THIS REPORT SHALL NOT BE REPRODUCED EXCEPT IN FULL WITHOUT THE WRITTEN AUTHORITY OF THE LABORATORY.
ALL SAMPLES WILL BE DISPOSED OF AFTER 30 DAYS FOLLOWING ANALYSIS. PLEASE CONTACT THE LAB IF YOU REQUIRE ADDITIONAL SAMPLE STORAGE TIME.

ALS LABORATORY GROUP ANALYTICAL REPORT

		Sample ID	L739693-1				
		Description					
		Sampled Date	04-MAR-09				
		Sampled Time	10:30				
		Client ID	HSDX09-SI-14				
Grouping	**Analyte**						
SOIL							
Physical Tests	Loss on Ignition @ 550 C (%)		13.6				
Particle Size	% Gravel (>2mm) (%)		1				
	% Sand (2.00mm - 1.00mm) (%)		<1				
	% Sand (1.00mm - 0.50mm) (%)		7				
	% Sand (0.50mm - 0.25mm) (%)		19				
	% Sand (0.25mm - 0.125mm) (%)		21				
	% Sand (0.125mm - 0.063mm) (%)		7				
	% Silt (0.063mm - 0.0312mm) (%)		11				
	% Silt (0.0312mm - 0.004mm) (%)		14				
	% Clay (<4um) (%)		19				

Reference Information

Additional Comments for Sample Listed:

Samplenum	Matrix	Report Remarks	Sample Comments

Methods Listed (if applicable):

ALS Test Code	Matrix	Test Description	Analytical Method Reference(Based On)
LOI-550-SK	Soil	Loss on Ignition @ 550 C	CSSS (1993) p.461-462

The dry-ash method involves the removal of organic matter by combustion at 550OC for 16 - 20 hours. Samples are dried prior to combustion.

Carter, Martin. Soil Sampling and Methods of Analysis. Can. Soc. Soil Sci.(1993) method 44.3.3

PSA-PIPET-DETAIL-SK	Soil	Particle size - Sieve and Pipette	FORESTRY CANADA (1991) P. 46-48 MOD

Particle size analysis involves the measurement of the proportions of the various primary soil particle sizes (ie. clay < 0.004 mm, silt 0.004-0.063 mm, sand 0.063-2.0 mm and gravel > 2.0 mm). In this method, the gravel and sand portions are determined by sieving, while the silt and clay portion is determined by sedimentation using Stokes Law, which relates the radius of the particles to the velocity of the sedimentation in water. Pretreatment of the soil with Calgon (sodium hexametaphosphate) is used to ensure the complete dispersion of the primary soil particles. Additional pretreatments may be necessary to remove cementing materials such as CaCO3 and organic matter.

Reference
Y.P. Kalra, and D.G. Maynard, 1991. Methods Manual For Forest Soil and Plant Analysis,Northwest Region. Forestry Canada (modified sand, silt and clay size ranges)

** Laboratory Methods employed follow in-house procedures, which are generally based on nationally or internationally accepted methodologies.
The last two letters of the above ALS Test Code column indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location	Laboratory Definition Code	Laboratory Location
SK	ALS LABORATORY GROUP - SASKATOON, SASKATCHEWAN, CANADA		

GLOSSARY OF REPORT TERMS
Surr - A surrogate is an organic compound that is similar to the target analyte(s) in chemical composition and behavior but not normally detected in enviromental samples. Prior to sample processing, samples are fortified with one or more surrogate compounds.
The reported surrogate recovery value provides a measure of method efficiency.
mg/kg (units) - unit of concentration based on mass, parts per million
mg/L (units) - unit of concentration based on volume, parts per million
N/A - Result not available. Refer to qualifier code and definition for explanation

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Although test results are generated under strict QA/QC protocols, any unsigned test reports, faxes, or emails are considered preliminary.

ALS Laboratory Group has an extensive QA/QC program where all analytical data reported is analyzed using approved referenced procedures followed by checks and reviews by senior managers and quality assurance personnel. However, since the results are obtained from chemical measurements and thus cannot be guaranteed, ALS Laboratory Group assumes no liability for the use or interpretation of the results.

Hatfield CONSULTANTS

Hatfield Consultants
201-1571 Bellevue Avenue
West Vancouver, BC V7V 1A6
1-604-926-3261

L739693

REPORT TO:

Company: Hatfield Consultants
Address: 201-1571 BELLEVUE AVE
West Vancouver, B.C.
V7V 1A6
Contact: NARA HENDERSON
Phone: 604-926-3261
Fax: 604-926-5389
Email: nhenderson@hatfieldgroup.com

INVOICE TO:

Company: Hatfield Consultants
Address: 201-1571 BELLEVUE AVE
West Vancouver, B.C.
V7V 1A6
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Phone: 604-926-3261
Fax: 604-926-5389
Email: nhenderson@hatfieldgroup.com

Project Number: HS 1489
Project Name: Howe Sound

Sampler's Name: NARA HENDERSON /Glen Bruce
Signature: Bruce

ANALYSIS REQUESTED

Client Sample ID	Matrix	Sampling Date	Sampling Time	Container Type/No.	Other	PARTICAL SIZE DETAILED Refer to Previous Hatfield Projects	%LOI			
HSDX09-SI-14	SOIL	4-Mar-09	10:30	Ziplock Bag						

Relinquished by (Signature): Bruce
Date: 5 March 2009 Time: 11:00

Received by (Signature): Mar 5/09
Date: Anna Time: 13:09

Courier | Waybill No.

Remarks: Soil Size Refer to Previous Hatfield Projects.

Cooler	
Temp (deg. C)	14°C
Custody Seal #	
Seal Intact Y/N	
Sample Tags Y/N	