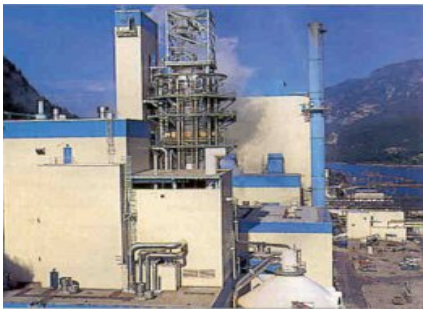
EFFECTS OF TWO INDUSTRIAL EFFLUENTS ON JUVENILE WHITE STURGEON (*Acipenser transmontanus)*

Teck Cominco



Celgar Pulp Limited

Prepared by:

Joy Bruno
Pacific Environmental Science Centre
Environment Canada

Prepared for:

Sturgeon Contaminants Working Group

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Acknowledgments

Joy Bruno prepared this report. The study was under the direction of Graham C. vanAggelen in support of the Upper Columbia River White Sturgeon Recovery Initiative (UCRWSRI) Contaminants Working Group Sub Committee.

The work presented was conducted at Environment Canada's Pacific and Yukon (P&Y) Regional Environmental Toxicology Laboratory, located at the Pacific Environmental Science Centre (PESC) in North Vancouver, British Columbia. Michelle Linssen, Craig Buday, Grant Schroeder, and Heather Osachoff provided assistance with the study. The Chemistry Section of the PESC laboratory provided sterol analysis.

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1.0 INTRODUCTION

The following report outlines and discusses work completed during the summer/fall of 2002 in support of the Upper Columbia River White Sturgeon Recovery Initiative (UCRWSRI) Contaminants Working Group Sub Committee. This project forms part of a multi-year investigation to be carried out on Upper Columbia River sturgeon (*Acipenser transmontanus*).

The UCRWSRI is a consortium of various U.S. and Canadian government agencies people, industry representatives, academics and consultants interested in, and working on the recovery of sturgeon populations in the Upper Columbia River between the Lower Arrow Lake in Canada and Lake Roosevelt above the Grand Coulee Dam in the U.S. One question the UCRWSRI Recovery Team members have been asking is "what role might pollution play in the lack of successful recruitment of Columbia River white sturgeon?". To answer this question, a technical group called the Sturgeon Contaminants Working Group was formed as a sub-committee to assist the Recovery Team. This group is attempting to characterize contaminant levels in Columbia River white sturgeon and examine the possible contribution contaminants might have in combination with other environmental stressors, in the lack of recruitment and survival of this population of sturgeon.

1.1 The Role of the Environmental Toxicology Laboratory

PESC environmental toxicology laboratory has a long established history of conducting toxicological tests on a variety of species. The laboratory is fully accredited for a number of toxicological tests and has a considerable amount of expertise in experimental design, animal husbandry and toxicological data review.

1.2 Study Objectives

It has been hypothesized that effluents from various industrial and municipal operations and non-point sources in the Columbia River are causing detrimental effects to Columbia River white sturgeon populations. In particular, the Upper Columbia River between the Hugh Keenleyside Dam at Castlegar and downstream to the U.S. border has several potential point sources of contaminants: three major treated sewage discharges, two at Castlegar and another just south of Trail; secondarily treated pulp mill waste from Celgar Pulp Company in Castlegar just downstream of the Hugh Keenleyside Dam; and process and cooling water from Teck Cominco Metals Ltd. (Teck Cominco), Trail Operations zinc/lead smelter.

The Sturgeon Contaminant Working Group is tasked with assessing contaminant levels in Columbia River white sturgeon to aid in the prevention of further reduction in this population. In order to assess the potential deleterious effects of effluents entering the Columbia River, effluent from both Teck Comino, Trail Operations and Celgar Pulp Company Ltd. was collected and used in two early life stage juvenile white sturgeon toxicity tests.

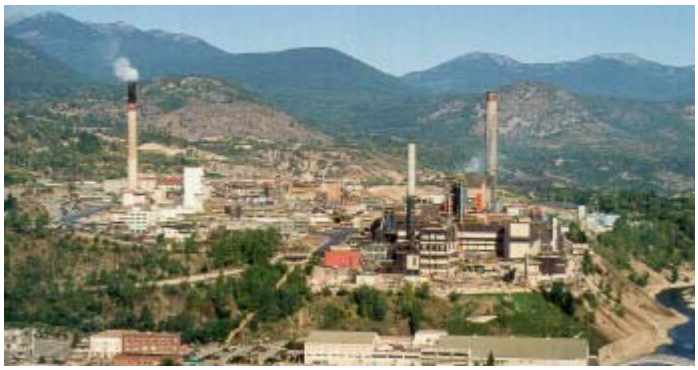
The toxicity tests using the early life stages of Columbia River hatchery white sturgeon were modeled after the Environment Canada method EPS 1/RM/28 Second Edition-July 1998 Biological Test Method: Toxicity Tests Using Early Life Stages of Salmonid Fish (Rainbow Trout). This document describes testing using the three major transitions in the development of early life stage of fish from egg to alevin to fry. For this study we chose the transitional period between yolk-sac fry to free swimming fry, roughly an 8-week exposure.

2.0 MATERIALS AND METHODS

2.1 The Effluent Sample Sites

2.1.1 Teck Cominco Metals Ltd.

Teck Cominco Metals Ltd. (Teck Cominco)'s Trail Operations are set within the boundaries of the City of Trail along side the Columbia River. The Trail Operations are



the world's largest fully integrated zinc and lead smelting and refining complex with production capacities of approximately 300,000 tonnes/year of zinc and 120,000 tonnes/year of lead. The Trail complex is also a significant producer of silver, gold, indium, germanium, bismuth, and copper products, including copper sulphate and copper arsenate. It also produces a large volume of sulphur products including ammonium sulphate fertilizer, sulphuric acid, liquid sulphur dioxide and elemental sulphur.

The majority of the zinc concentrates treated at Trail come from the Red Dog Mine in Alaska and the remainder from other mines in the U.S. and Canada. Lead concentrates are purchased from mines in the U.S. and South America. The company's Waneta Hydroelectric Dam located nearby on the Pend d'Oreille River generates power for the metallurgical operations.

Most of the major production plants at Trail were modernized between 1977 and 1997 at a cost of over $1 billion. Further upgrades are ongoing as new technologies emerge and equipment ages resulting in higher productivity and improved environmental performance. During this study the average flow from EW 87 (CSO-III) was 100,000 m^3/day.

2.1.2 Celgar Pulp Company Ltd.

The Celgar pulp mill is located on the Columbia River just west of the town of Castlegar, BC.

Celgar Pulp is recognized as a producer of 2 grades of quality Northern Bleached Softwood Kraft pulps (Celstar and Celect) made from a unique blend of slow growing/long fiber Western Canadian tree species. They produce 1200 tons per day. Celstar is made from a balance of Hemlock, Balsam Fir, Spruce, Pine and Western Red Cedar. Celstar is typically used in coated publication grades, special printing and writing grades. Celect is made from a specially segregated mixture of long fiber wood species (Douglas Fir and Western Larch). Celect is used in newsprint, ground specialty, tissue and supercalendered grades.

In 1989 the mill was substantially rebuilt to increase production capacity and bring the mill into full compliance with existing and expected environmental requirements. The modernization project was completed in 1993 at a cost of $800 Million. Celgar's effluent treatment system consists of: solid removal in the primary clarifier, effluent cooling, biological aeration for 48 hours, and secondary clarification. This removes up to 95% of the BOD (biological oxygen demand), and the low level of chlorinated organics (AOX) is

further reduced by 40% before discharge to the Columbia River. Process flows to the effluent treatment system average 66,500 m^3 per day. The treated effluent is combined with clean cooling water prior to discharge to the Columbia River. The average flow rate of this combined discharge is approximately 125,000 m^3/day.

2.2 Effluent Collection

As Teck Cominco was in the process of a summer maintenance shutdown, effluent to be used for the entire eight week study period was collected from Combined Sewer Outfall 3 (CSO III) over a 4-day period (July 31st, August 6th, 7th, 8th 2002). CSO III is one of Teck Cominco's permitted compliance points prior to effluent entering the Columbia River. Thirty 40 litre sealed drums were collected and shipped to A-1 Cold Storage, Surrey BC until required to refresh the test.

Effluent was collected weekly (grab sample) from the secondary foam tank at Celgar Pulp Company Ltd from July 30 to September 17, 2002. Effluent collection at Celgar occurred at the permit compliance point where the effluent is diluted with cooling water and is the last point before entry into the Columbia River underwater diffuser.

Effluent was shipped (via either Van-Kam Freightways Limited, Westarm Truck Lines, or Nickel's Cartage Company Limited) and arrived at either A-1 Storage or in the laboratory at PESC within 48 hours of collection. Once at the laboratory the barrels collected that week were combined and homogenized into one large sample. The composite was then distributed into 300 -L barrels and stored at 4 ± 2°C until required for refreshment of the solutions throughout the following week. For each collection of effluent from either Teck Comino or Celgar sub samples were removed from the containers and allowed to come to test-specific temperature as needed for use in each of the series of biological tests.

2.3 Suite of Toxicity tests

Concurrent to the ELS test, a number of other toxicity tests were performed for further effluent toxicity profiling. The initial sample of effluent was tested for acute lethality to rainbow trout underyearling using a static 96-hr exposure-LC_{50} method, and for metabolic inhibition of a bioluminescent bacterium, *Vibrio fischeri,* using an acute 5- and 15-min toxicity testing (IC_{50}) protocol. Routine water quality measurements were taken during the toxicity tests (pH, dissolved oxygen, temperature, conductivity). Weekly samples for sterol analysis were conducted on the effluent from Celgar.

2.4 The Acute Lethality Test Using Rainbow Trout

The potential for effluent to cause acute toxicity was determined using the standards 96 hour LT50 rainbow trout, *Oncorhynchus mykiss*, bioassay. Effluents were tested at the 100% concentration using testing procedures outlined by Environment Canada (1990, 2000). The test was started on either Thursday or Friday (effluent delivery day) the static

(no solution renewal) 96hr-LT_{50} test (time to 50% mortality of the test population) was set-up in a temperature-controlled environmental chamber set to maintain a temperature of 15 ± 1°C and a photoperiod of 16hr lightness and 8hr darkness with a morning and evening transition time of 15 minutes. These conditions coincided with those in the fish acclimation/holding area of the laboratory.

For the test, test vessels (beakers or aquaria) were rinsed with control/dilution water which was the laboratory supply of groundwater (hardness, ~100 mg/L $CaC0_3$) obtained from the well built on the Pacific Environmental Science Centre site. The temperature of the freshwater supplied to each testing room can be controlled on a room-by-room basis, and was set at 14 ± 1°C for the 96 hr-LC_{50} test.

Oil-free compressed air was delivered to each test vessel at a rate of 6.5 ± 1 mL/min by means of aquarium airline tubing and disposable borosilicate glass Pasteur pipettes. Test solutions were preaerated (i.e., before exposure to fish) for 30 min upon which the dissolved oxygen (D.O.) level was measured. After pre-aeration, underyearling rainbow trout fry were placed into the test vessels. Mortality and behavior were observed periodically throughout the test, more frequently during the first few hours and daily until test end, after 96 hours of exposure.

2.5 The Acute Toxicity Test Using a Photoluminescent Bacterium

Applying the Microtox™ test system, a marine bioluminescent bacterium, *Vibrio fischeri*, which emits a blue green light at 490 nm was used to assess the toxicity of the effluent. Vials of freeze-dried *V. fischeri* stored at -20 ±2°C were reconstituted in 1.0 mL of distilled water and incubated at 5.5 ± 1 °C for no less than 20 minutes prior to use in liquid-phase tests. Test results were based on measured light output in the presence of various levels of test substance in aqueous solutions, which were compared with light output of a control blank (i.e. bacterial cell suspension in diluent only). Light output is a product of the electron transport system and relates directly to the metabolic state of the bacteria (Schiewe, M.H., E.G. Hawk, D.I. Actor and M.M. Krahn et al.1985). The degree of light loss (degree of metabolic inhibition in the bacteria) indicates the sample's toxicity.

The initial sample of effluent from Teck Cominco and weekly effluent samples from Celgar were tested on either the day of its delivery or the next day for toxicity. The effluent sample was tested using liquid-phase testing procedures IC_{50}-determination as outlined by Microbics Corporation (1992) and Environment Canada (1992). A 2% NaCl crystal - distilled water solution was used as control and diluent water during liquid-phase testing. Light emission readings were recorded after 5 and 15 minutes of incubation at 15.0 ± 0.5 °C in controls and test solutions.

A Microtox™ model 500 Toxicity Analyzer controlled by the appropriate Microtox™ Omni software for Windows® (version 1.15) was used for liquid-phase acute testing procedures. Following exposure to toxic samples, this software also reduces the light

reading data to determine a dose-response curve on which the IC_{50} is located (a 95% confidence range is then also reported). The IC_{50} is the inhibiting concentration of a sample causing a 50% decrease in the bacterial light output under defined conditions of exposure time and test temperature.

2.6 Early Life Stage Toxicity Testing Using Developing White Sturgeon

Pursuant to British Columbia Regulation 261/83 made under the Wildlife Act (Section 110(2)(h)(aa)) permission was granted to possess and transport live freshwater fish (sturgeon) within the Province of British Columbia to PESC (Transplant License 9623). White Sturgeon originating from Columbia River between Hugh Keenleyside Dam and United States border residing at Hill Creek Hatchery, Nakusp, BC were used as brood stock. Sturgeon hatched from July 23rd-26th 2002 were used in the study. On July 31st, three thousand 5-8 day post hatch juvenile sturgeon were transferred from Hill Creek Hatchery in Nakusp, British Columbia to Kelowna, BC and then flown to Vancouver and couriered to PESC arriving on August 2nd, 2002.

Sturgeon were acclimated to laboratory water by placing the 7-10 day old sturgeon contained in large plastic bags with water in two large aquaria (100 and 200 liter capacity) and the water was allowed to come to aquaria temperature (14°C). After one hour, one liter of aquaria water was added to the holding bags and the waters were allowed to mix, this procedure was repeated after another hour. At then end of the second hour the sturgeon were released into the two aquaria. The fish were held in the aquaria until they were used in testing, four days later. Each day during the acclimation the water in the aquaria was changed 50% with test control/dilution well water.

The temperature in the environmental chambers dedicated to ELS testing was set to uniformly maintain 14 ± 1°C. Lighting was the same as that for acclimation (<500 lux at the surface) with a photoperiod (a light:dark cycle of 16± 1 h : 8± 1 h). Test vessels consisted on 35 L glass aquaria. The laboratory well water served as control/dilution water; a single set of fresh solutions for all replicates was prepared in a 200 L vat. Seven replicates for each solution were set up: three replicates were used to harvest sturgeon every five days during the study, and four replicates were used for mortality, and behavioral and physiological observations. The concentrations of effluent used in testing were 100%, 50% (Teck Cominco only), 1%, and 0%.

The volume of solution required for the weekly exchanges/replenishments were determined by the estimated growth fish size and corresponding loading density (not to exceed 0.5 g/L, Appendix Table 1). A static solution renewal system was employed whereby solutions were renewed three times a week (Mondays, Wednesdays, and Fridays).

Solution renewal was achieved by siphoning out ~80% of the old solution and replacing it immediately with a fresh (new) temperature adjusted solution prepared to the same strength. Self-priming and submersible pumps were used to siphon 48-72 h-old solutions and to add freshly prepared solutions in the test vessels. Developing sturgeon lay

relatively undisturbed during the daily activities, as care was taken to avoid disturbing them. Gentle aeration was provided by bubbling oil-free compressed air through a clean disposable glass pipette. All solutions were aerated throughout the test, at a controlled rate of 6.5 1 ml/min.

To begin the test, on August 6th 2002, all solutions were preaerated (i.e., before exposure to fish) for 30 minutes upon which time the dissolved oxygen (D.O.) level was measured. After pre-aeration, a small volume and mesh size net was used to transfer the approximately 11-14 days- old juvenile sturgeon from the holding aquaria into large sized weigh boats containing ~ 200 mL of the appropriate test solution which corresponded to each replicate vessel. Using this regime, the sturgeon were immediately exposed to the test solutions of various concentration while the correct numbers of sturgeon were counted into each aquaria. There were seven replicates per test solution each containing approximately 65 developing sturgeon. Other water quality parameters measured at the test start included pH, temperature and conductivity.

During the testing, daily observations were made to ensure dead organisms were removed from solutions. Numbers of dead and any deformed/abnormal juveniles were recorded on the analyst log. Organisms were removed from the sampling replicates (E, F,G) at day 5, and every fifth day until the end of the test. Immediately upon the fishes removal from the test exposure, sturgeon were anesthetized using MS-222 (100 mg/L). These samples were archived for gene expression profiling (Appendix, Table 2).

Feeding began when the juveniles began to exhibit swim-up behavior. Sturgeon were fed #2 BioDiet Starter (Bio-Oregon, Inc. Warrenton, Oregon); this feed was provided by the Ministry of Water, Land and Air Protection. The hatchery recommended ration level was 10.61% of wet body weight per day as based on the total biomass of sturgeon in each tank. Based on known growth trajectories for this ration level, the quantity of food was adjusted weekly to reflect the change in biomass. However, this proved to be too much food in a static renewal system and fungi build-up became a problem therefore sturgeon were fed ad lidum three times a day. This could explain in part why the actual measured growth was well below the estimated growth.

The duration of the study was from 11-14 days post hatch to 61-64 days post hatch. At test end surviving fry were examined for deformities/abnormalities and archived for future gene expression profiling. Final water quality was also determined at this time.

2.8 Sterol Analyses

Weekly sub samples of effluent from Celgar Pulp Company were submitted to the PESC Chemistry laboratory for chemical analysis to their sterol content. Sterols were analyzed using solid phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS). One liter samples were preserved, pH adjusted to 3 then filtered twice (1.2 μm, 0.45 μm). Sterols were extracted from the solid phase and concentrated, then they were back extracted with ethyl acetate and the sample was cleaned up using silica gel. Next it was

acetylated using pyridine/acetic anhydride and extracted using petroleum ether. The sample was concentrated and analyzed using GC/MS.

3.0 RESULTS

3.1 Short-term Biological Tests

Table 1 summarizes the results of the toxicity tests conducted during the ELS tests

Table 1. Toxicity Results from suite of tests associated with Sturgeon Exposures

Week	Test	100% Celgar	100% Teck Cominco
1	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	not acutely toxic
	96 hr. Rainbow Trout Beaker Test	0/2 dead	2/2 dead within 48 hrs
	96 hr. Rainbow Trout Aquaria Test	not done	1st Barrel 1/10; 2nd Barrel 3/10 dead
2	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	No need to test further since will
3	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	only be using these barrels for testing
	96 hr Beaker Test (Rbt/Sturgeon)	0/5 dead Rbt; 0/2 dead Sturgeon	only 1% continued to be used in testing
4	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	
	96 hr Beaker Test (Rbt/Sturgeon)	0/5 dead Rbt; 0/3 dead Sturgeon	
5	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	
	96 hr. Rainbow Trout Beaker Test	0/5 dead	
6	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	
	96 hr. Rainbow Trout Beaker Test	0/5 dead	
7	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	
	96 hr. Rainbow Trout Beaker Test	0/5 dead	
8	Microtox, 5 and 15 Liquid Phase Test	not acutely toxic	
	96 hr. Rainbow Trout Beaker Test	0/5 dead	

These results indicate that the effluent from Celgar Pulp Company was not acutely lethal (no fish died during testing, Microtox indicated effluent was not acutely lethal) while the effluent from Teck Cominco was not toxic to Photoluminsecent bacteria but had some toxicity to rainbow trout. The 100% concentration of effluent from TeckComino was acutely lethal to rainbow trout; for the 1st screen of the effluent both trout died within 48 hours of exposure. The effluent was re-tested and only one rainbow trout out of 10 died during this 96-hour exposure, another test using another barrel resulted in 3 out of 10 rainbow trout dying during the exposure period.

3.2 Early Life Stage Development Test

The ELS test was terminated on Day 50, on the eighth week of exposure. Mean (and standard deviation of the mean) pH, dissolved oxygen, temperature and conductivity is presented in Table 2 (all water quality measurement are presented in the Appendix, Table 3). Control/dilution water quality of 24 h to 72 h old and freshly prepared solutions was relatively consistent at levels (pH: 7.0-7.8; D.O.: 7.3 mg/L - fully saturated; temperature

12.7-15.2). Full strength (100% v/v) effluent from Teck Cominco, Trail Operations had a pH range of 7.3 to 7.9; a D.O. from 8.0 to fully saturated: and a temperature range from 14.3 to 16.2; none of these measurements are considered detrimental to fish health. For the 100% Celgar effluent pH levels ranged from 7.3 to 8.7; D.O. ranged from 6.9 to fully saturated; while temperature ranged from 13.8 to 16.4 °C none of these measurements are considered detrimental to fish health.

Table 2. Water Quality Data- Control, Teck Cominco and Celgar Pulp

	TECK COMINCO					CELGAR PULP		
	Well	**1%**	**50%**	**100%**		**Well**	**1%**	**100%**
pH								
mean	7.3	7.4	7.5	7.6		7.3	7.3	8.3
SD	0.2	0.2	0.1	0.2		0.1	0.1	0.3
range								
Low (minimum)	7.0	7.1	7.4	7.3		7.0	7.1	7.3
High (maximum)	7.8	7.8	7.7	7.9		7.6	7.6	8.7
DO								
mean	9.2	9.1	9.5	9.5		9.2	9.0	9.1
SD	0.7	0.8	0.5	0.9		0.6	0.7	0.8
range								
Low (minimum)	7.3	7.5	8.5	8.0		7.3	7.5	6.9
High (maximum)	10.2	10.2	10.0	10.3		10.2	10.1	10.1
Temperature								
mean	13.8	14.6	14.8	15.2		13.9	14.6	15.3
SD	0.6	0.6	0.4	0.7		0.7	0.6	0.8
range								
Low (minimum)	12.7	12.7	14.3	14.3		12.7	12.8	13.8
High (maximum)	15.0	15.8	15.4	16.2		15.2	16.1	16.4
Conductivity								
mean	436	456	567	653		436	460	1524
SD	20	23	29	5		20	29	146
range								
Low (minimum)	410	420	520	650		410	410	1200
High (maximum)	480	500	610	660		480	520	1690

The rainbow trout ELS test (Environment Canada 1998) is valid if at test end less than 40% of the controls are nonviable. Although there is no comparable validity test for tests using sturgeon, the controls associated with both the Teck Cominco and Celgar Pulp Company effluents were below 40% at 38.4 ± 29.4 % and 36.1 ± 7.5%, respectively.

3.2.1 ELS test Endpoints:

For the full strength Teck Cominco maintenance-period effluent, the LT_{50} was 4 days (96 hours), while for the half-strength effluent, the LT_{50} was 7 days. All sturgeon exposed to the 100% effluent were dead after 5 days of exposure while all sturgeon in the 50% effluent died within 17 days. The 50% effluent exposure used the same batch of sturgeon but was set up on day 3 of the study therefore day 20 is the 17^{th} day of their exposure to the effluent. There was an attempt to impede the mortality of the sturgeon exposed to the

100% effluent by removing a subpopulation and placing them in control/dilution water. These fish however did not recover from their 3-day effluent exposure and all died after 17 days.

Toxicity effects were not evident after exposure to any of the tested concentrations (100%, 1%) of the Celgar Pulp Company effluent. The LT_{50} for sturgeon mortality (%) was not determinable as less than 50% of the fry died in any of the treatments.

There was no increased incidence of abnormal behavior in the 1-100% concentrations as compared to the control. Exposure to all concentrations of the effluent (1-100%) resulted in a low incidence of alevin and fry deformity. For those treatments for which survival was adversely affected (i.e. 100% effluent with a survival rate of 35% or lower), delayed development and/or mortality likely precluded the discovery of any physical abnormalities. Average weight of sturgeon taken during the exposure indicates that there was not a significant difference between the controls and the treatments.

3.3.1 Sterol Analysis

The sterol compounds contained in the collections for Celgar Pulp Company are displayed in Table 3. Beta-sitosterol levels ranged from 0.15 to 1.04 ug/L and stigmasterol ranged from .06 to .49 ug/L. Dark green boxes represent the highest value for that sterol while the light green boxes represent the lowest value for that particular sterol in this data set.

Table 3. Celgar Pulp and Foam Tank Sterol Results

												Range	
Sterols	**WK1**	**WK2**	**WK3**	**WK4**	**WK5**	**WK6**	**WK7**	**WK8**	**Units**	Average	SD	Low	High
17alpha-estradiol	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
17alpha-ethynylestradiol	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	ug/L	< 0.02	0	< 0.02	
17beta-estradiol	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
5a-cholestane surrogate	51	50	24	25	21	112	55	>40	%	47.25	29.4	21	112
beta-sitosterol	0.243	0.349	0.155	0.201	0.488	1.040	0.383	0.242	ug/L	0.363	0.311	0.155	1.040
Campesterol	0.052	0.108	0.031	0.045	0.136	0.238	0.051	0.037	ug/L	0.0872	0.0713	0.031	0.238
Cholesterol	0.121	0.154	0.057	0.058	0.076	0.098	0.038	0.044	ug/L	0.0875	0.0407	0.057	0.154
Coprostanol	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
Desmosterol	0.013	0.016	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	n/a	n/a	< 0.005	0.016
Dihydrocholesterol	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
Epicoprostanol	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
Equilin	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
Equol	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	ug/L	< 0.2	0	< 0.2	
Estriol	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	ug/L	< 0.02	0	< 0.02	
Estrone	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
Mestranol	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ug/L	< 0.005	0	< 0.005	
Norethindrone	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	ug/L	< 0.2	0	< 0.2	
Norgestrel	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	ug/L	< 0.2	0	< 0.2	
Stigmasterol	0.064	0.230	0.101	0.268	0.488	0.162	0.229	0.033	ug/L	0.197	0.145	0.064	0.488

3.4 Chemical Analysis

The 100% Teck Cominco effluent was analyzed for total metals; the values were: zinc ranged from 128 to 273 ug/L (average 166), lead ranged from 12.5 to 54 ug/L (average 21.6), copper ranged from1 to 6 ug/L (average 2.5) and cadmium ranged from 2.28 to 3.35 ug/L (average 2.55). The zinc, lead and cadmium values are above the Canadian Environmental Quality Guidelines for water: aquatic life freshwater (zinc 30 ug/L, lead 1-7 ug/L, copper 2-4 ug/L and cadmium 0.2-1.8 ug/L).

The chemistry results for the 100% Celgar are: resin and fatty acids and PAHs (polycyclic aromatic hydrocarbons) levels were low while organohalides (AOX) were 0.87-2.0 mg/L; total metals for Celgar were low except for weeks 6 and 7 when the zinc levels were higher than other weeks (7.1-33 ug/L) at 176 and 141 ug/L, respectively; only 5 of the 18 sterols analyzed for had levels above the detection limits. The top three sterols were: β sitosterol ranging from 0.15 to 1.04 ug/L, Stigmasterol from 0.06 to 0.49 ug/L, and Campesterol 0.03-0.24 ug/L.

4.0 DISCUSSION

4.1 The Acute Toxicity of Effluent

The acute toxicity tests (96-hr LT50, Microtox) indicate that the effluent collected from Teck Cominco July 31 – August 8, 2002 was not toxic to Photoluminescent bacteria but had some toxicity to rainbow trout fry. Acute toxicity test results with effluent from Celgar Pulp Company collected July 30 – September 17, 2002 indicate that the effluent was not acutely lethal to either photoluminescent bacteria or rainbow trout fry.

4.2 The Toxicity of Industrial Effluents to White Sturgeon

This study demonstrated that exposure to effluent from a zinc/lead smelter was capable of causing mortality to developing sturgeon. This observation was only manifested in sturgeon exposed to the 50 and 100% effluent; it was not observed when fish were exposed to the 1% effluent. Successful reproduction of fish populations requires the successful development of offspring into new reproductive cohorts. The results of this project determined that the ELS white sturgeon were sensitive to the Teck Cominco effluent in the laboratory and indicate that long-term exposure to the effluent sample may present potential problems to developing fish.

5.0 CONCLUSIONS

Concentrations of 100% and 50% concentrations of the Teck Cominco effluent collected for this study were lethal to juvenile sturgeon, however sturgeon exposed to 1% concentrations of the effluent did not exhibit significantly higher mortality than the control. There was no significant difference in mortality between juvenile sturgeon exposed to well water control, 1% and 100% effluent from Celgar Pulp Company.

Based on the results of the beaker test it appears that the sturgeon are more sensitive to the Teck Cominco effluent than rainbow trout although this might be a matter of sensitive life stage. Rainbow trout were underyearlings typically the size used in acute toxicity tests (average wet weight 0.3-2.5 g) whereas the sturgeon were only 11-14 to 32-35 days old (average weight .07-.16 g)

6.0 RECOMMENDATIONS

These tests are only the 1st stage of the report, future analysis will consist of using the molecular tools to compare tissue from exposed organisms to unexposed or control organisms to determine differences in gene expression levels attributed to the contaminants in the effluents. An interpretive report will be prepared to evaluate the ELS bioassay results with effluent chemistry and genomic results.

This study examined only two of the identified potential sources of contaminants into the Columbia River; other possible sources of contaminants should be studied. There is also the need to verify laboratory results with those found in the field. Further studies should also include in-situ tests with eyed eggs in the Columbia River to see if they elicit the same responses.

Complex mixtures such as those found in treated pulp mill and lead/zinc smelters effluents may have subtle and complex effects on fish reproductive physiology. In many cases, dilutions of these complex mixtures in the environment are below the threshold for observation of these effects. However, the potential for environmental effects remains at sites with insufficient or seasonally low dilution rates. Precisely what the ecological relevance of these subtle effects are remains to be seen. Continued study will ultimately elucidate information that can result in the reduction of deleterious effects to the white sturgeon.

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APPENDIX

Table 1. Volumes of Effluent required for weekly refreshes of White Sturgeon Early Life Stage Test

Week	Age of Fish (days old)	Total volume per replicate per replicate in Liters	~ Volume required per effluent per change in Liters	Volume required per week in Liters
1	7 to 13	10.1	71	213
2	14 to 20	20.2	141	424
3	21 to 27	30.3	212	636
4	28 to 34	35.4	250	743
5	35 to 41	40.4	282	848
6	42 to 48	50.5	353	1060
7	49 to 55	50.5	353	1060
8	56 to 62	50.5	353	1060

Table 2. Sampling Schedule for Juvenile White Sturgeon
Sturgeon Fish Collections August-September 2002

Vial Identification 2002	Contents	Date Collected	Day Collected	Comments
S1	Control-Celgar	August 11th	5	
S2	1% Celgar	August 11th	5	
S3	100% Celgar	August 11th	5	
S4	Control-Teck Cominco	August 11th	5	
S5	1% Teck-Cominco	August 11th	5	
S6	100% Teck-Cominco	August 11th	5	
S7	Control-Celgar	August 16th	10	
S8	%1 Celgar	August 16th	10	
S9	100% Celgar	August 16th	10	
S10	Control-Teck Cominco	August 16th	10	
S11	1% Teck-Cominco	August 16th	10	
S12	50% Teck-Cominco	August 16th	10	
S13	Control-Celgar	August 21st	15	
S14	%1 Celgar	August 21st	15	
S15	100% Celgar	August 21st	15	
S16	Control-Teck Cominco	August 21st	15	
S17	1% Teck-Cominco	August 21st	15	
S18	100% Teck-Cominco	August 21st	15	Probably 50% not 100%
S19	Control-Celgar	August 26th	20	
S20	%1 Celgar	August 26th	20	
S21	100% Celgar	August 26th	20	
S22	Control-Teck Cominco	August 26th	20	
S23	1% Teck-Cominco	August 26th	20	
S24	50% Teck-Cominco	August 26th	20	
S25	Control-Celgar	August 31st	25	
S26	%1 Celgar	August 31st	25	
S27	100% Celgar	August 31st	25	
S28	Control-Teck Cominco	August 31st	25	
S29	1% Teck-Cominco	August 31st	25	
S30	100% Teck-Cominco	August 31st	25	All fish dead not done
S31	Control-Celgar	September 5th	30	
S32	%1 Celgar	September 5th	30	
S33	100% Celgar	September 5th	30	
S34	Control-Teck Cominco	September 5th	30	
S35	1% Teck-Cominco	September 5th	30	
S35	100% Teck-Cominco	August 31st	25	All fish dead not done
S37	Control-Celgar	September 10th	35	
S38	%1 Celgar	September 10th	35	
S39	100% Celgar	September 10th	35	
S40	Control-Teck Cominco	September 10th	35	
S41	1% Teck-Cominco	September 10th	35	
S42	100% Teck-Cominco	August 31st	25	All fish dead not done
S43	Control-Celgar	September 15th	40	
S44	%1 Celgar	September 15th	40	
S45	100% Celgar	September 15th	40	

S46	Control-Teck Cominco	September 15th	40
S47	1% Teck-Cominco	September 15th	40
S48	50% Teck-Cominco	September 15th	40
S49	Control-Celgar	September 20th	45
S50	%1 Celgar	September 20th	45
S51	100% Celgar	September 20th	45
S52	Control-Teck Cominco	September 20th	45
S53	1% Teck-Cominco	September 20th	45
S54	100% Teck-Cominco	September 20th	45
S55	Control-Celgar	September 25th	50
S56	%1 Celgar	September 25th	50
S57	100% Celgar	September 25th	50
S58	Control-Teck Cominco	September 25th	50
S59	1% Teck-Cominco	September 25th	50
S60	50% Teck-Cominco	September 25th	50

# of samples to process

54

Table 3. Water Quality Measurements

pH				DO				Temperature				Conductivity			
TECK COMINCO				TECK COMINCO				TECK COMINCO				TECK COMINCO			
Well	1%	50%	100%	Well	1%	50%	100%	Well	1%	50%	100%	Well	1%	50%	100%
7.3	7.3		7.6	9.6	9.8		9.9	14.0	13.8		14.8	440	470		650
7.4	7.6		7.6	9.5	9.9		9.9	14.3	14.4		14.8				
7.3	7.4		7.6	10.2	9.8		10.2	15.0	14.8		15.2	440	470		650
7.4	7.5		7.6	10.2	10.2		10.3	14.5	14.2		16.2	440	470		650
7.6	7.8	7.7	7.3	9.0	9.2	8.5	8.0	14.5	14.6	14.3	14.3				
7.2	7.3	7.5	7.9	8.8	9.7	9.5	8.6	13.5	15.4	15.4	16.0	450	470	580	660
7.4	7.3	7.4	Down	9.5	9.5	9.5	Down	12.7	15.2	15.1	Down	440	480	560	Down
7.4	7.3	7.4		10.0	9.8	9.6		13.5	14.8	15.0		430	450	560	
7.7	7.6	7.6		9.0	8.6	9.7		14.3	15.0	14.6					
7.4	7.3	7.6		10.1	9.9	10.0		13.7	12.7	15.0		410	430	520	
7.3	7.4	7.5		10.0	10.1	9.8		13.4	14.6	14.5		480	490	610	
7.3	7.4	7.5		9.2	9.1	9.1		13.7	14.3	15.1		440	450	570	
7.7	7.6	7.6		9.8	9.6	10.0		14.4	14.6	14.6					
7.3	7.3	Down		8.6	9.0	Down		13.2	14.9	Down		460	460	Down	
7.1	7.1			9.3	9.1			13.4	14.8			460	480		
7.1	7.3			8.6	8.0			13.1	15.8			460	500		
7.4	7.2			8.4	7.6			14.2	14.4						
7.2	7.3			8.9	9.0			13.2	14.1			460	470		
7.1	7.2			8.3	9.6			13.3	15.4			440	480		
7.2	7.2			9.7	9.5			13.7	13.9			410	440		
7.2	7.5			7.3	9.2			14.9	14.4						
7.3	7.4			8.7	8.9			13.4	14.6			430	450		
7.2	7.1			9.8	9.2			13.3	14.6			420	430		
7.2	7.1			8.7	8.8			13.7	15.1			420	430		

	7.8	7.8			9.6	10.0			14.6	14.5						
	7.2	7.4			9.6	9.4			13.6	14.8			420	420		
	7.1	7.1			8.4	7.7			13.2	14.7			410	430		
	7.0	7.3			9.5	9.0			13.4	14.8			410	430		
	7.2	7.4			8.3	7.7			14.7	14.3						
	7.2	7.3			9.7	8.7			13.3	14.8			420	430		
	7.5	7.2			9.1	7.5			14.4	14.3						
av	**7.3**	**7.4**	**7.5**	**7.6**	**9.2**	**9.1**	**9.5**	**9.5**	**13.8**	**14.6**	**14.8**	**15.2**	**436**	**456**	**567**	**653**
SD	**0.2**	**0.2**	**0.1**	**0.2**	**0.7**	**0.8**	**0.5**	**0.9**	**0.6**	**0.6**	**0.4**	**0.7**	**20**	**23**	**29**	**5**
range																
low	**7.0**	**7.1**	**7.4**	**7.3**	**7.3**	**7.5**	**8.5**	**8.0**	**12.7**	**12.7**	**14.3**	**14.3**	**410**	**420**	**520**	**650**
high	**7.8**	**7.8**	**7.7**	**7.9**	**10.2**	**10.2**	**10.0**	**10.3**	**15.0**	**15.8**	**15.4**	**16.2**	**480**	**500**	**610**	**660**

	pH			**DO**			**Temperature**			**Conductivity**		
	CELGAR PULP MILL			**CELGAR PULP MILL**			**CELGAR PULP MILL**			**CELGAR PULP MILL**		
	Well	**1%**	**100%**	**Well**	**1%**	**100%**	**Well**	**1%**	**100%**	**Well**	**1%**	**100%**
	7.3	7.3	7.6	9.4	9.6	9.8	15.2	13.8	13.8	440	460	1630
	7.4	7.4	8.3	9.1	9.2	10.0	14.7	14.4	14.2			
	7.3	7.4	8.4	10.2	10.1	10.1	15.0	14.9	15.5	440	460	1630
	7.2	7.3	8.4	10.2	9.2	9.8	15.0	15.2	16.0	440	460	1630
	7.6	7.4	8.0	9.0	8.8	8.5	14.5	14.5	14.3			
	7.2	7.2	7.3	8.8	9.1	8.1	13.5	15.2	15.7	450	460	1550
	7.4	7.4	8.4	9.5	9.7	9.7	12.7	14.7	16.4	440	470	1570
	7.4	7.2	8.4	10.0	9.7	9.8	13.5	14.9	15.5	430	460	1540
	7.6	7.6	8.3	9.2	9.0	9.5	14.5	14.4	14.3			
	7.3	7.3	8.6	9.6	9.8	9.7	13.9	12.8	16.3	410	420	1570
	7.3	7.3	8.5	10.0	9.7	9.9	13.4	14.7	16.0	480	520	1680
	7.3	7.4	8.5	9.2	9.3	9.4	13.7	14.1	14.4	440	460	1500
	7.3	7.3	8.3	8.7	9.8	8.4	14.4	14.4	14.4			
	7.3	7.3	8.3	8.6	9.0	8.2	13.2	14.9	16.1	460	480	1580
	7.1	7.3	8.4	9.3	9.6	9.5	13.4	14.9	15.9	460	500	1510
	7.1	7.2	8.5	8.6	7.9	8.3	13.1	16.1	16.0	460	500	1650
	7.1	7.2	8.0	8.9	8.9	9.4	14.6	14.4	14.3			
	7.2	7.4	8.6	8.9	8.6	9.2	13.2	15.0	16.1	460	490	1690
	7.1	7.2	8.7	8.3	9.2	8.7	13.3	15.2	15.1	440	480	1610
	7.2	7.4	8.5	9.7	8.8	9.0	13.7	14.0	15.5	410	410	1600
	7.2	7.2	7.9	7.3	8.2	7.7	14.5	14.5	14.9			
	7.3	7.4	8.6	8.7	8.8	8.9	13.4	14.6	16.1	430	440	1570
	7.2	7.3	8.5	9.8	9.7	9.7	13.3	14.6	15.9	420	430	1520
	7.2	7.1	8.3	8.7	8.7	8.9	13.7	15.1	15.9	420	440	1530
	7.6	7.5	8.4	9.6	9.1	9.4	14.6	14.5	14.6			
	7.2	7.2	8.6	9.6	8.8	9.5	13.6	14.8	16.1	420	430	1200
	7.1	7.1	8.4	8.4	7.8	8.9	13.2	14.7	15.9	410	440	1250
	7.0	7.1	8.3	9.5	9.0	9.6	13.4	14.2	14.3	410	430	1260
	7.2	7.2	7.9	8.3	7.7	6.9	14.7	14.6	14.5			
	7.2	7.3	8.6	9.7	8.7	9.3	13.3	15	16.2	420	490	1260
	7.3	7.1	8.0	9.1	7.5	8.2	14.6	14.4	14.5			
av	**7.3**	**7.3**	**8.3**	**9.2**	**9.0**	**9.1**	**13.9**	**14.6**	**15.3**	**436**	**460**	**1524**
SD	**0.1**	**0.1**	**0.3**	**0.6**	**0.7**	**0.8**	**0.7**	**0.6**	**0.8**	**20**	**29**	**146**

range												
low	**7.0**	**7.1**	**7.3**	**7.3**	**7.5**	**6.9**	**12.7**	**12.8**	**13.8**	**410**	**410**	**1200**
high	**7.6**	**7.6**	**8.7**	**10.2**	**10.1**	**10.1**	**15.2**	**16.1**	**16.4**	**480**	**520**	**1690**