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Assessment of Industrial Effluent Toxicity Using Flow-Through Fish Egg/Alevins/Fry (EAF) Toxicity Test

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The overall objective of this study was to determine if adding small amounts of seawater would detoxify the effluent of an aluminum smelter. A previous TIE study had identified aluminum as a possible source of the occasional acute toxicity to rainbow trout. The toxicity of aluminum to rainbow trout decreases with water hardness (CCREM, 1997), and seawater into which the effluent discharges was the nearest source of a hardening agent. The conditions which caused high concentrations of aluminum were storm events which were frequent in Kitimat, British Columbia (>200 in. per year). It was postulated that by adding small amounts of seawater (less than would be considered a substantial dilution factor) the increased hardness of the effluent would make it acutely non toxic to rainbow trout. The purpose of this study was: 1) to evaluate the Environment Canada's Embryo/Alevin/Fry (EAF) method; 2) to determine the suitability of the early life stage test for assessing the quality of Alcan's effluent, and 3) to assess the toxicity of Alcan's effluent after treatment with small amount of seawater. In this paper, the acute and chronic effects of an aluminum smelter effluent using rainbow trout (*Oncorhynchus mykiss*) in the EAF Toxicity Test were investigated. This work was completed at BC Research Inc. (BCRI).

MATERIALS AND METHODS

Rainbow trout (*Oncorhynchus mykiss*) eggs and milt were supplied by a local hatchery from three females and three males. The gametes were dry fertilized on the day of receipt prior to introducing them into the appropriate test concentrations. Each control and treatment had four replicates with 100 eggs in each replicate. The number of organisms was culled to 30 after the swim-up stage was reached.

The effluent and storm runoff from Alcan Smelters and Chemicals Ltd. in Kitimat, BC, flow into and are collected in the Upper B-Lagoon. This lagoon has a short holding time depending on the storm event. The effluent samples were taken where the Upper B-Lagoons discharges to the Lower B-Lagoon. Samples of seawater were collected from water pumped up from the wharf. Approximately

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600 to 100 litres of effluent were shipped twice a week in 1 m^3 HDPE tanks to the laboratory.

The test vessels consisted of a food grade polyethylene 7-L pails. Masterflex pumps were used to supply a flow rate of 6.5 litres of solution per day. Oil-free compressed air was supplied from the BCRI main air compressor through standpipes in each test chamber through disposable Pasteur pipettes.

At the start of the test the laboratory was kept in darkness, except for dim lighting when the technician was preparing solutions, checking on mortalities, and taking water quality measurements. One week after all eggs had hatched the lights were turned on with a 16 hr light and 8 hr dark photoperiod and with an average intensity of 130 lux. The temperature was kept at 10°C until 50% of the eggs had hatched when it was increased to 12°C. When the fish had reached the swim-up stage the temperature was increased to 15°C. The eggs and alevins were kept in the Nalgene basket suspended in the test solutions. When the alevins began the swimup stage some escaped from the baskets and moved into the pails. Later all fry were released into the pails to allow better observation of swimup behavior. Fry were fed small amounts of finely ground starter trout chow several times a day to determine when they were ready to start feeding. When feeding behavior started they were fed 4% of their body weight divided into five feedings per day. After two weeks it was noted that the more aggressive fish were eating all of the food so fewer larger feedings were offered to allow the smaller fish access to some food.

Every day pH, dissolved oxygen, temperature and flow rate were measured in a representative replicate of each treatment. Daily observations were made in every replicate for mortalities, deformities, behavior and, at the appropriate times, hatching and swimup behavior. The conductivity of each solution was measured after mixing to ensure that the correct proportions were used. At the end of the test, the percent mortality and dry weight of each fish were recorded in each replicate and analyzed for significant difference from the controls.

The mean (±SD) of non-viable embryos and dead alevins was calculated for each control and treatment group. The mean values for the replicates of the treatment group were then compared statistically with corresponding values for the controls. Initially, homogeneity of variances for replicated treatments was tested (Eisenhart et al. 1947; Sokal and Rohlf 1969) and data transformation applied if necessary (Mearns et al. 1986). One-Way ANOVA was used for the multiple comparison, followed by Dunnett's test or Tukey's test or other suitable procedure for multiple comparisons of each treatment group versus the control.

The basic protocol for this study was the Environment Canada RM series "Toxicity Tests using Early Life Stages of Salmonid Fish (Rainbow Trout, Coho Salmon, or Atlantic Salmon), August and December 1996 draft versions (Environment Canada 1996). The flow-through option was used, mixing fresh solution every other day from effluent and seawater which was shipped twice a

week. The major changes to this protocol were the treatments which were chosen to be tested. Instead of testing a range of concentrations of the effluent diluted with lab water, three different amounts of seawater were added to the effluent, 1.25%, 2.5% and 5%. The 100% effluent was also used throughout the test. Three different controls were run concurrently, using BCRI laboratory dechlorinated and hardened Vancouver city water and laboratory water with 2.5% seawater added. The tests were done in food grade approved polyethylene pails. To determine if the plastic could effect the growth and development of young fish another control was set up using glass aquarium and laboratory water.

Each batch of effluent and effluent with 2.5% seawater was analyzed for dissolved metals, anion scan, total cyanide, alkalinity, dissolved organic carbon, and total mercury. Hardness, pH, and conductivity were analyzed in the 100% effluent. An acute rainbow trout mortality was done on each batch of effluent at 100%, 98.75%, 97.5%, and 95% concentrations diluted with seawater.

The test began on December 9, 1996 when all eggs had been distributed to the appropriate solutions. This was done within 30 minutes after the dry fertilization period. The embryo stage continued 30 d until 250% had hatched on January 13, 1997. All replicates hatched within one day of each other. The alevin stage continued for 19 d, until February 1, when 250% of the alevins in the controls were exhibiting swim-up behavior, i.e. maintaining their position in the water column and actively feeding. All other replicates achieved the swim-up stage within 49 d and all replicates were fed from Feb. 1 until March 5, 1997. The test continued until all replicates had been fed for 30 d. They were not fed for the last 24 hours of the test. The test ended on March 6, 88 d after the test began and the fish were sacrificed and dried for weight comparisons.

Every batch of effluent was sub sampled at its source and 20-40 L was air freighted to BCRI to arrive one day ahead of the tote. A preliminary 96 hr acute rainbow trout bioassay was done at 100%, 98.75%, 97.5% and 95% using seawater to dilute the sample. If it was determined that a particular batch was acutely lethal it was not used for the 100% concentration in the EAF test but the previous effluent was used until the next shipment arrived. These tests were done according to the standard Environment Canada protocol “Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout” (Environment Canada, 1990).

RESULTS AND DISCUSSION

The HDPE 1 m^3 totes used for transporting effluent were proved to be acutely non-toxic. All control groups were statistically non-different in all the endpoints being observed, indicating that neither the use of the plastic pail nor the addition of small amount of seawater (2.5% v/v) was contributing to the adverse effect detected in the experiment.

The average percentage of viable control embryos was ≥ 70%. Unfertilized eggs were included in the count of non-viable control embryos when the estimation was made. The percent nonviable embryos did not exceed 15% in any of the controls or treatments. There were no more than 1 or 2 deformed alevins or fry in any replicate. The coefficient of variation of individual dry weights for all surviving control fish, measured at the end of the test was 23%, less than 30% required.

Under three circumstances the 100% effluent failed the 96 hr LC50 rainbow trout test. This occurred when the aluminum concentration in the effluent was 3.63, 2.4, and 5.1 mg/L. When the effluent was mixed with seawater at 98.75:1.25 (v/v), 97.5:2.5 (v/v) or 95:5 (v/v), fish survival rates increased to 79-90%.

Using even 1.25% seawater to treat the effluent seemed to significantly reduce the lethality of the effluent. All acute fish bioassays passed an LC50 test in the seawater treated effluent groups.

Between fertilization and 50% hatching out, there was no significant difference in mortality among all control and treatment groups, indicating that the toxic effluent did not have a significant impact on the newly fertilized eggs (Figure 1).

After 50% eggs were hatched, fish in 95% and 98.75% effluent groups started to show higher mortality if compared with the controls. Since the acutely toxic effluent with dissolved aluminum concentration above 2 mg/L was not used in the 100% effluent group, fish mortality in this group was not statistically different from the controls (Figure 1).

Acutely toxic effluent received shortly before 50% swim-up fry stage contributed to the very high mortality in all seawater treatment groups (Figure 1). Accumulative mortality in the seawater treated effluent groups were significantly higher than the controls when 50% swim-up stage was reached (Figure 1). At the end of the test, fish body weight was significantly higher in the control groups than in the 100%, 98.75%, 97.5 and 95% effluent groups (Figure 2), indicating the growth of the fry was inhibited by the sublethal exposure to the effluent.

One of the major objectives of this study was to find out whether the addition of small amount of seawater could alleviate the acute effluent toxicity. The four incidences of high aluminum concentration (> 2.0 ppm) in the effluent occurred on Dec.4, 1996, Jan. 9, Jan. 29, and Feb. 19, 1997, coinciding with survival rates below 50% in the acute screening bioassays. However, all seawater treated effluent passed the acute toxicity test, indicating that fish survival increases significantly with as little as 1.25% v/v seawater addition (Figure 1). It became apparent that the seawater addition could detoxify the effluent so that none of the 96-hr acute toxicity bioassay tests would fail. Effluent characterization confirmed that the lethal toxicity of the effluent was due to the high concentration of dissolved aluminum at relatively low hardness and slightly acidic pH.

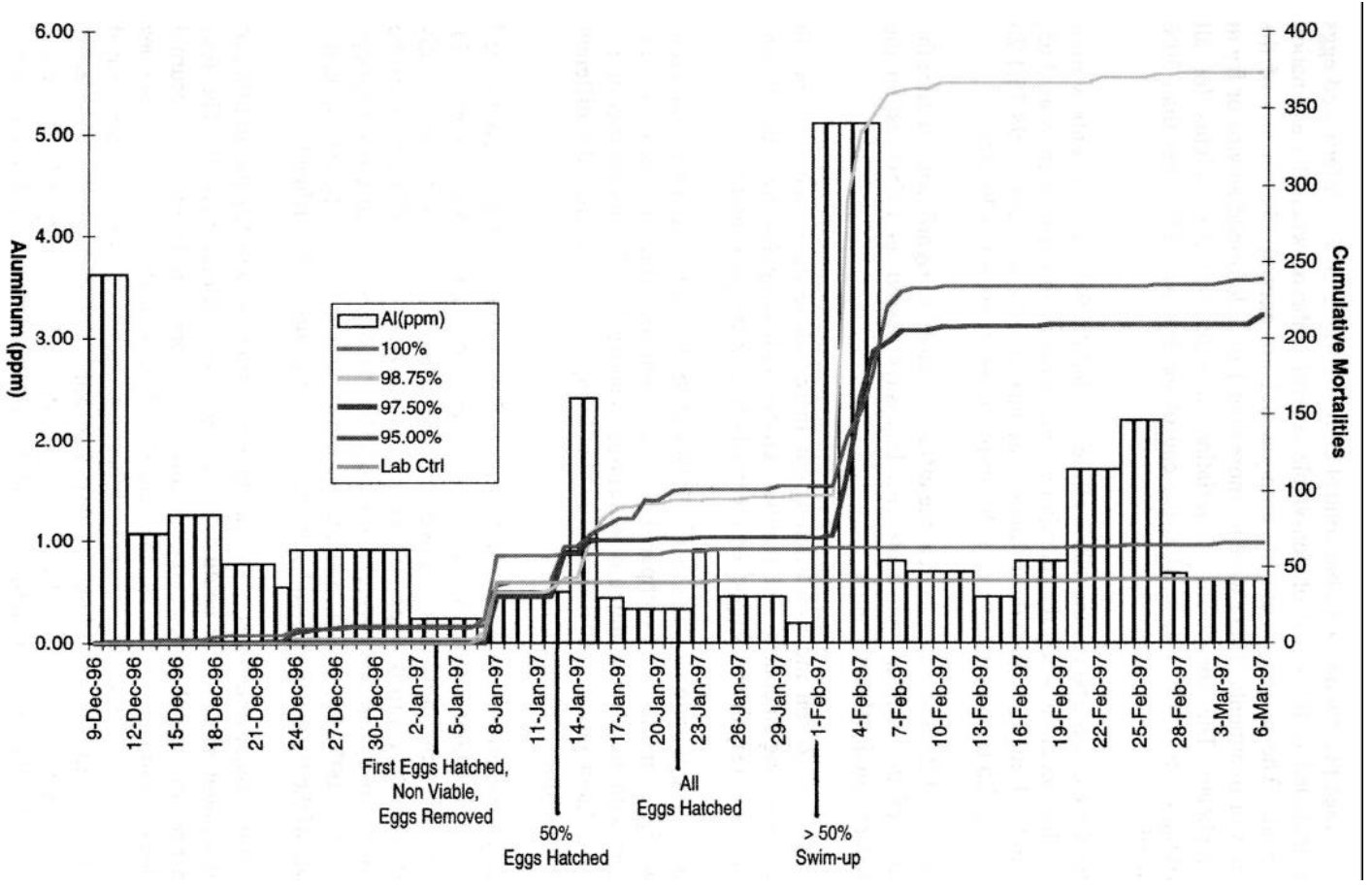

Figure 1. Accumulative mortality throughout the test and effluent aluminum concentrations.

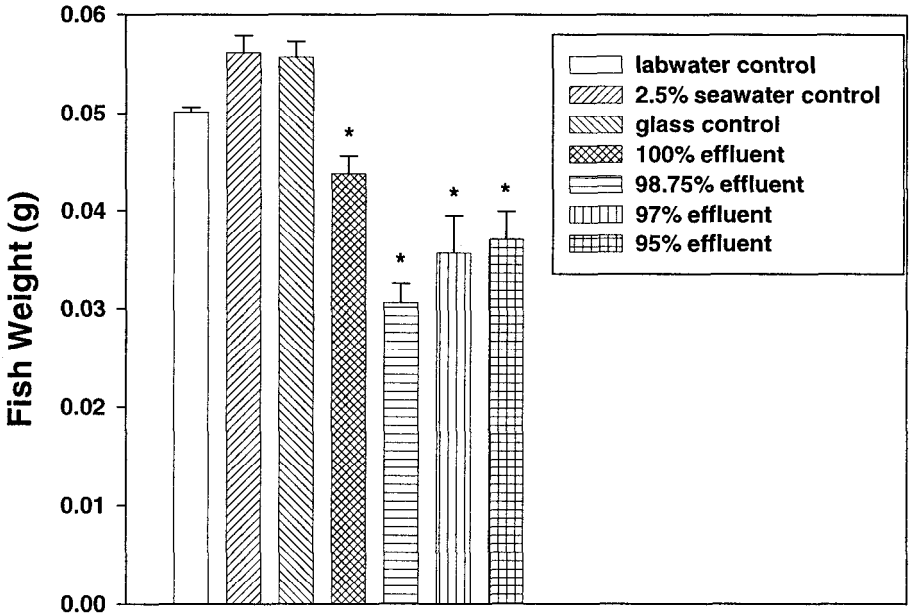

Figure 2. Effect of effluent exposure on fish growth in the EAF test. Asterisk indicates statistical difference from control ($p<0.05$).

Although acute toxicity could be avoided by treating the effluent with seawater, in the EAF test the overall accumulative mortality in the 1.25%, 2.5 % and 5% seawater groups were found to be significantly higher than the controls around the 50% swim-up transition period and at the end of the whole experiment (Figure 1). Note that the 100% treatment did not use effluent which was acutely toxic to rainbow trout, but this effluent was used for all seawater treatments.

The mortality could result from various environmental factors such as pH, temperature and salinity (Larson et al. 1977). Since seawater was added to three effluent groups, one control group was designed with 2.5% seawater mixed with laboratory water to determine if there was an effect of seawater addition on the early life stage development. It was shown that the seawater control was no different in the endpoints from other control groups, meaning seawater addition was not likely a confounding factor in the endpoint observations.

The higher mortality in the seawater-treated effluent groups could be mainly related to the elevated concentrations of aluminum in the effluent since the concentrations of all other chemicals, including metals and cyanide, were fairly low. In addition, it has been shown that during the initial water hardening stage the egg lacks sensitivity to cyanide (Woltering 1984). The relatively high sensitivity of the early embryo to chemical harm may rest with the process of water hardening, because the intake of water may also bring into the egg some of

the dissolved chemicals such as aluminum. Exposure of Atlantic salmon eggs to 1mM aluminum (valence 3) caused a complete cessation of water hardening while cations of lesser charge, such as zinc, also cause some reduction in water uptake when tested at the same concentration but did not block the process entirely.

The acutely toxic effluent received shortly after 50% swim-up fry stage obviously contributed to the very high mortality in all seawater treatment groups (Figure 1, suggesting that aluminum was mainly responsible for the effluent toxicity. After the swim-up fry stage, virtually no mortality was observed even in the 100% effluent group. Overall, the accumulative mortality in the seawater-treated effluent groups was significantly higher than the controls when 50% swim-up stage was reached (Figure I). These toxic events occurred at possibly the sensitive stages, when fish just emerged from the protection of the egg shell and when they were about to swim up, during which time the yolk sac was absorbed and the active feeding had not yet started. Since fish were vulnerable during the transition stages when the toxic events occurred, the deaths were speculated to be largely related to the effluent toxicity breakthroughs rather than the variation in the sensitivity of the development stage.

A commercial starter feed suitable for rainbow trout fry was used and the fry were fed 4% of their body weight per day, with approximately equal portions of this ration offered five times per day for the first 15 d. Due to apparent unequal feeding behavior and mortality in the control from possible malnourishment, feeding for the last 15 d was done three times per day with a medium, small and large feeding in the afternoon. Any delay in making food available to the fry was avoided wherever possible in order to reduce the possibility of stress from starvation. It was also implemented to synchronize the amount of food relative to the rate of development and number of fish in each replicate. As such, food availability was assumed to be the same among all groups during the test.

At the end of the test, fish dry body weight was found to be significantly higher in the control groups than in the 10098.75%, 97.5% and 95% effluent groups (Figure 2), indicating the growth of the fry was inhibited by the sublethal exposure to the effluent. Although other components in the effluent could also be related to the decrease in fish fry body weight at the end of the 88 d experiment, it seemed that aluminum might be the major reason for the sublethal toxicity observed. The mechanism of the aluminum sublethal toxicity on growth is complicated. The fish growth data tends to suggest that even though the addition of seawater may solve the problem in effluent acute toxicity, long-term effects on fish growth may still exist.

The EAF test was found to be a sensitive biological tool for toxicity assessment. Compared with the life cycle tests that include mature fish, the EAF test qualifies as a quicker and less expensive means of estimating the chronic toxicity of test substances or effluents. However, like most sublethal studies of fish, it requires the maintenance of the animals for months in the lab under well-defined

conditions. The long duration increase the likelihood of mechanical or power failure or disease ruining the whole test. On the other hand, due to the nature of the flow-through bioassay, the daily parameter measurement and condition checking and maintenance (e.g. peristaltic pump flow rate adjustment, removing non-viable eggs, etc.) makes the test fairly labor-intensive and therefore costly. Extra care should be taken when these experiments are carried out since the basis of utilizing fish early life stage test is their sensitivity.

When industrial effluent is evaluated, these tests are more suitable for on-site bioassays because the shipment of the effluent and the QA/QC processes associated with effluent transportation and storage can make the cost much higher. As a result, the EAF test is more appropriate for non-routine toxicity identification evaluation rather than routine bioassays.

The results indicated that the addition of seawater to the effluent rendered it non acutely toxic to rainbow trout. When effluents with high aluminum concentration (> 2 mg /L) were removed from the 100% effluent treatment, it was shown that low concentrations did not have an adverse effect on mortality or growth.

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