QUANTITATIVE EXPOSURE-RESPONSE MODEL FOR MORTALITY IN BROWN TROUT FRY EXPOSED TO ZINC

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1.0 INTRODUCTION

1.1 Background

The upper Arkansas River in the vicinity of Leadville, Colorado, has been impacted by releases of mining-related contamination from the Leadville mining district. This impact has been primarily due to releases of dissolved and suspended heavy metals into the river via California Gulch, which is one of the main drainages for the Leadville mining district (Figure 1-1). The metal of chief concern to aquatic receptors in the upper Arkansas appears to be zinc, with a smaller impact contributed by cadmium (EPA 2004a).

In the past, mining impacts caused or contributed to a reduction in the density of fish (brown trout) in the river below the confluence with California Gulch. The USEPA has been working to control and remediate sources of contamination in the Leadville mining district, and in recent years the level of contamination released from California Gulch into the upper Arkansas appears to have been decreasing. At the same time, fish density has been tending to increase at stations below California Gulch such as AR-3A, AR-4 and AR-5 (see Figure 1-2). AR-1 has been included in the figure as a reference location because it is upstream of California Gulch. This is consistent with the hypothesis that remedial actions at the site have resulted in improved water quality and increased aquatic health, but further analysis is required to determine if this apparent trend is related to site remediation or to other variables. One approach for further investigation is through development and application of quantitative methods for predicting mortality in fish based on measured water quality measurements.

1.2 Methods for Predicting Toxicity Based on Water Quality Data

Toxicity of metals such as zinc depend on two key variables: the concentration of zinc, and the hardness of the water (increasing hardness tends to decrease toxicity). One way to account for both hardness and zinc concentration in the prediction of toxicity is to develop a dose-response curve where the percent mortality is plotted as a function of the Hazard Quotient (HQ), which is calculated as:

\[
HQ_{C,H} = \frac{C}{TRV_H}
\]

where:

- \(HQ_{C,H}\) = Hazard Quotient at a zinc concentration of C and a hardness of H
- C = Concentration of zinc (ug/L)
- H = Hardness of the water (mg/L)
- TRV_H = Toxicity Reference value for zinc at hardness H
Basis of Hardness-Dependent TRVs

Studies performed by CDOW indicate that the TRV$_H$ depends upon the developmental history of the exposed organism: fry that have not been pre-exposed to zinc ("non-acclimated") tend to be more sensitive than those that have been pre-exposed ("acclimated") during their early life stages (ELS). For the purposes of this assessment, attention is focused on data for non-acclimated fish. This approach is likely to provide some margin of safety with regard to any fry in the river that are acclimated.

Based on a large number of acute mortality studies in brown trout, CDOW found that the LC50 for non-acclimated fry exposed to zinc at hardness $H$ is given by the following equation:

$$LC50_H (\text{ug/L}) = \exp[2.679 + 0.9634 \cdot \ln(H)]$$

The acute TRV at hardness $H$ is equal to $\frac{1}{2}$ the LC50$_H$:

$$\text{Acute TRV}_H (\text{ug/L}) = \frac{LC50_H}{2} = \exp[1.986 + 0.9634 \cdot \ln(H)]$$

Data on chronic toxicity of zinc on brown trout are less extensive. Based on several 90 day studies, CDOW previously derived the following equation for the chronic TRV as a function of $H$:

$$\text{Chronic TRV}_H (\text{ug/L}) = \exp[0.9805 \cdot \ln(H) + 1.402]$$

More recently, CDOW has proposed that the chronic TRV for zinc in brown trout be derived from the more robust acute data set by applying an acute-chronic ratio (ACR) to the acute equation:

$$\text{Chronic TRV}_H (\text{ug/L}) = \frac{LC50_H}{\text{ACR}}$$

The ACR recommended by CDOW is 2.5, which based on an extensive literature report of ACR values for zinc in a number of different species (Canton 2003). This value was reviewed and approved by Colorado's Water Quality Control Commission in 2004. This results in the following:

$$\text{Chronic TRV}_H (\text{ug/L}) = \exp[1.846 + 0.9634 \cdot \ln(H)]$$

Figure 1-3 compares the chronic TRV values predicted by the older approach and the proposed new approach. As seen, the values are relatively close together. For the purposes of this report, the TRVs used to calculated HQ values are based on the proposed new approach.

Fitting Mortality Data to an Exposure-Response Model

Given a set of toxicity data where percent mortality is reported as a function of the HQ for zinc (calculated as above), it is necessary to fit the data to a mathematical model, as follows:
Response (% mortality) = Bkg + (1-Bkg)Φ[k1 + k2·ln(HQ)]

where:

Bkg = Average response (% mortality) in control organisms
Φ = standard normal cumulative distribution function

The parameters of the model (k1 and k2) are found by minimization of squared errors. Given the parameters of the equation, the expected percent mortality in brown trout fry can be calculated for any sample of water where the zinc concentration and water hardness are known.

2.0 OVERVIEW OF ZINC TOXICITY STUDIES IN BROWN TROUT

USEPA has performed several studies to investigate the toxicity of water from California Gulch and the Arkansas River on brown trout fry, and the Colorado Department of Fish and Wildlife (CDOW) has performed a related series of laboratory studies intended to provide additional data on zinc toxicity to brown trout. The purpose of this report is to summarize studies performed by USEPA and CDOW in 2004 and 2005, and to utilize these data to develop a quantitative model for predicting mortality as a function of water quality measurements. Attachment A contains the HQ calculations for each study.

2.1 USEPA FIELD STUDIES

2.1.1 2004 Flow Through Serial Dilution Study

A detailed description of the 2004 study design is provided in EPA’s Quality Assurance Project Plan (QAPP) for the project (USEPA 2004b).

The test organisms were brown trout (Salmo trutta). Eyed eggs were received on 10/24/03 from the Colorado Division of Wildlife Research Hatchery in Bellevue, CO. These eggs were collected as part of an annual CDOW spawning operation in Delaney Buttes Reservoir, CO. Eggs were placed in a 5-gallon aquarium containing 5 L of water that was prepared by mixing well water and reverse osmosis water to yield a hardness of about 130 mg/L. Temperature was controlled with an incubator bath to be about 3-5°C. Measured water quality parameters are summarized below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>132 mg/L</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>95.1 mg/L</td>
</tr>
<tr>
<td>DO</td>
<td>10.3 mg/L</td>
</tr>
<tr>
<td>pH</td>
<td>8.02</td>
</tr>
<tr>
<td>Temperature</td>
<td>4.9°C</td>
</tr>
</tbody>
</table>
Each day, 75% of the water in each aquarium was replaced. Aquariums were cleaned as needed, and dead organisms were removed and counted weekly. Upon swimup (03/05/04, 133 days after receipt of eggs), fry were transferred to 10-gallon flow-through aquariums, which received 100 mL/min chilled water at the same concentrations as above. Fry were fed trout chow (Silver Cup) four times daily (twice daily on weekends and holidays) at an estimated rate of 1% body weight/day. Trout chow was initially supplemented with <24 hr old brine shrimp nauplii to encourage feeding.

About two weeks before exposure studies began, the hardness of the water was gradually reduced by increasing the proportion of reverse osmosis water and decreasing the proportion of well water to yield a target hardness of about 50 mg/L. This is a typical hardness at Station AR-3 when the flow through study took place.

Exposure of the fry began on 05/20/04 (76 days post swimup). At the time of testing, the average length of the fry was 32± 2.8 mm, and the average fry weight was 0.248 ± 0.0278 g. The study utilized a flow-through design using water derived from California Gulch Station CG-4 ("source water") and water derived from the upper Arkansas River Station AR-1 ("dilution water"). Both the source water and the dilution water were collected daily and placed in large holding tanks, which were used to supply the exposure tanks. Table 2-1 presents water quality parameters for the source water (CG-4) and the dilution water (AR-1) on each day of the study. As seen, concentration values fluctuated only slightly during the time of the study.

The source water and the dilution water were combined using serial dilutors (1:3) to produce waters of the following compositions:

<table>
<thead>
<tr>
<th>Water Source</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tank 1</td>
</tr>
<tr>
<td>Source Water (CG-4)</td>
<td>100%</td>
</tr>
<tr>
<td>Dilution Water (AR-1)</td>
<td>0%</td>
</tr>
</tbody>
</table>

Measurements of water temperature, pH, conductivity, and dissolved oxygen in the exposure tanks were taken daily during the study, and samples for the analysis of inorganics, hardness (by titration), and alkalinity were collected at the beginning and the end of the study. Water samples for dissolved organic carbon (DOC) analysis were taken on day 7 of the sampling period. Water quality results for the exposure tanks are shown in Table 2-2. Concentrations values measured in water from AR-3A during the same time frame (5/20/04 to 5/28/04) are very similar to the highest dilution water in the on-site study (Table 2-3).

At each dilution, two replicate exposure chambers were used. Each chamber contained 10 fish (a total of 20 for each fish type for each dilution). Exposure occurred for seven days. Fry were fed a mixture of trout chow (Silver Cup) four times daily (twice daily on weekends and holidays) at an estimated rate of 1% body weight/day. The diluter system was shut down for 15 - 30 minutes while the fish were fed. The number of fish alive and dead in each tank was recorded daily.

**Results**
Figure 2-1 presents the observed mortality data and corresponding HQ values for each dilution used in the 2004 flow-through study. As seen, mortality occurred in fry exposed to 100% California Gulch water, but there was no mortality in fry at any other dilutions.

2.1.2 2005 Flow-Through Serial Dilution Study

A detailed description of the 2005 study design is provided in EPA’s Quality Assurance Project Plan (QAPP) for the project (USEPA 2005).

The test organisms were brown trout (*Salmo trutta*) between 15 and 30 days post yolk sac adsorption with individual weights of at least 0.40 grams wet weight. Exposure of the fry began on 5/21/05 and ended on 5/27/05. Prior to the start of the study, the fry were maintained in an aquarium with flow-through conditions with a water hardness around 50 mg/L. A stainless steel heat exchanger maintained temperature at a mean temperature of 3.6 C for about 140 days. The temperature was increased at a rate of 1 degree Celsius per day to 12 degrees Celsius and maintained at 12 degrees Celsius for 14 days prior to the toxicity tests. The photoperiod consisted of 12 hours of light and 12 hours of dark. Fry were fed trout chow (Silver Cup) four times daily (twice daily on weekends and holidays) at an estimated rate of 1% body weight/day. Trout chow was initially supplemented with <24 hr old brine shrimp nauplii to encourage feeding.

The study utilized a flow-through design using water derived from California Gulch Station CG-4 (“source water”) and water derived from the upper Arkansas River Station AR-1 (“dilution water”). Both the source water and the dilution water were collected daily and placed in large holding tanks, which were used to supply the exposure tanks. Table 2-4 presents a summary of the measures water quality parameters in the holding tanks on each day of the study. As seen, concentration values fluctuated only slightly during the time of the study. The source water and the dilution water were combined using serial dilutors (1:2) to produce waters of the following compositions:

<table>
<thead>
<tr>
<th>Water Source</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tank 1</td>
</tr>
<tr>
<td>Source Water (CG-4)</td>
<td>100%</td>
</tr>
<tr>
<td>Dilution Water (AR-1)</td>
<td>0%</td>
</tr>
</tbody>
</table>

Measurements of water temperature, pH, conductivity, hardness, dissolved organic carbon (DOC), alkalinity, sulfate, chloride, and dissolved oxygen in the dilution tanks were taken daily during the study. Water quality results for the dilution tanks are shown in Table 2-5.

The flow-thru study was performed in accord with the basic methods detailed in the standard operating procedure (SOP) for chronic flow-thru bioassay with rainbow trout (see Attachment B) with necessary modifications for brown trout. At each dilution, two replicate exposure chambers were used. At the start of the study each chamber contained 15 fish (a total of 30 for each dilution). At the end of day 1, 5 of the 15 fish (all were alive at the end of day 1) were removed for the purpose of measuring zinc concentration bound to the gill. For the remaining 10 fry in
each chamber, exposure occurred for an additional six days (seven days total). The number of fry alive in each tank was recorded on each day of the study.

Results

Figure 2-2 presents the observed mortality data and corresponding HQ values for each dilution. HQ values are based on the proposed chronic CDOW parameters. As seen, a relatively steep increase in mortality occurs over the HQ range from 1.7 to 3.1, although one data point (HQ = 2.9) has an observed mortality (26%) that lower than expected from the other groups (60-70%). The cause of this lower than expected mortality is not known, but may simply be due to random variation.

2.1.3 2005 Zinc Spiking Study

In 2005, USEPA also performed a study in which Arkansas River water from Station AR-1 (upstream of California Gulch) was amended by addition of known levels of zinc. A detailed description of the 2005 study design is provided in EPA's Quality Assurance Project Plan (QAPP) for the project (USEPA 2005). The spiking study had the same design as the flow-through study described above, with a few modifications as summarized below.

Spiking Procedure

The highest concentration exposure water (referred to as “100%”) was prepared by combining AR-1 water at a flow rate of about 160 mL/min with a dosing pump that added a reagent grade zinc sulfate (ZnSO₄·7H₂O) at a concentration of 42.221g/20 L at a rate of 2 mL/min. This dosing solution was made by Dr. Steve Brinkman at CDOW. The target concentration of zinc in the 100% water was 6000 ppb. The 100% water was then diluted in a serial dilution system to relative concentrations of 50%, 25%, 12.5%, 6.25% and 0%.

Measurements of water temperature, pH, conductivity, hardness, dissolved organic carbon (DOC), alkalinity, sulfate, chloride, and dissolved oxygen in the dilution tanks were taken daily during the study. Water quality results for the dilution tanks are shown in Table 2-6.

Fish Removal

As above, after the first day, some of the 15 fish were removed for the purpose of measuring zinc concentration bound to the gill. However, because mortality was observed on day 1 in some tanks, the number of fish removed was adjusted to account for this initial mortality. The number removed are shown in Table 2-7. For the fish that remained, exposure occurred for an additional six days (a total of seven days). Fry were fed a mixture of trout chow (Silver Cup) four times daily (twice daily on weekends and holidays) at an estimated rate of 1% body weight/day. The diluter system was shut down for 15 - 30 minutes while the fish were fed.

Results
Table 2-7 lists the number dead on day 1 and day 7 as well as the number of fish removed for the zinc gill testing study. From these values the percent mortality was calculated using the following equation.

\[
\text{% Mortality} = \frac{d_1 + d_2 + r \left( \frac{d_2}{N_0 - d_1 - r} \right)}{N_0}
\]

where:
- \(d_1\) = number of fish dead on day 1
- \(d_2\) = number of fish dead on the last day
- \(r\) = number of fish removed for gill testing on day 1
- \(N_0\) = number of fish at the start of the study = 15

Figure 2-3 presents the mortality data and corresponding HQ values for each dilution used in the zinc spiking study. HQ values are based on the proposed chronic CDOW parameters. As seen, this study yielded a relatively smooth dose-response curve, with mortality occurring in the HQ range from 1-10.

2.2 CDOW LABORATORY STUDIES

2.2.1 2004 Laboratory Water Spiking Study

The laboratory study performed by CDOW in 2004 used a flow-through design and serial dilutions (1:2) to produce exposure waters. The source water was a mixture of well water and reverse osmosis water mixed to yield a hardness of about 50 mg/L. To this was added stock solutions prepared by dissolving reagent grade zinc sulfate (ZnSO\(_4\) 7H\(_2\)O) in deionized water. The water was chilled to 10°C and a recirculating water pump was used to aerate the water and to stabilize the pH. Measured water quality parameters for the laboratory exposure tanks are shown in Table 2-8.

Two replicate tanks containing 10 fish each were used per exposure group. Fry were fed four times daily (twice daily on weekends and holidays) at an estimated rate of 1% body weight/day. The diluter system was shut down for 15 - 30 minutes while the fish were fed. The number of fish alive and dead in each tank was recorded daily.

Figure 2-4 presents the mortality data and corresponding HQ values for each dilution used in the 2004 lab flow-through study. HQ values are based on the proposed chronic CDOW parameters. As seen, this study yielded a relatively smooth dose-response curve, with mortality occurring in the HQ range from about 3 to 15.

2.2.2 2005 Laboratory Water Spiking Study

In 2005, CDOW performed a laboratory study that was generally similar to the study performed in 2004. Dilution water consisted of a mixture of reverse osmosis water and water from a well
that is located onsite at the CDOW Aquatic Toxicology Laboratory. Waters were mixed to achieve a conductivity that provides water hardness similar to AR-3A during the time of in field studies (approximately 50 mg/L). Conductivity was controlled using Oakton conductivity controller. Egg and sac-fry incubated at 3 degrees Celsius until they reached a mass of approximately 0.4 g wet weight. The tests were conducted at 12 degrees Celsius. Prior to the initiation of the tests, sac-fry were warmed 1 degree Celsius per day to slowly acclimate fish to test temperatures. A re-circulating water pump was used to aerate source water and to stabilize water chemistries. Measured water quality parameters for the laboratory exposure tanks are shown in Table 2-9.

**Spiking Solutions**

Spiking stock solutions were prepared by dissolving a calculated amount of reagent grade zinc sulfate (ZnSO₄ .7H₂O) (Mallinkrodt) in de-ionized water. The initial (12 degree fish) were exposed to a zinc concentration of 6000 ppb (referred to as 100% water), and this water was diluted 50% serially to yield exposure levels of 50%, 25%, 12.5%, 6.25%, and 0%. New stock solutions were prepared as needed during the toxicity tests. Except for source water, spiking procedures and the number of replicate exposure chambers, the experimental operations were equal to those performed for EPA’s 2005 in-situ field toxicity test.

**Results**

Figure 2-5 presents the mortality data and corresponding HQ values for each dilution used in the 2005 lab spiking study. HQ values are based on the proposed chronic CDOW parameters. As seen, this study yielded a relatively smooth dose-response curve, with mortality occurring in the HQ range from about 1 to 10.

**3.0 COMPARISON OF FIELD AND LABORATORY STUDIES**

An important issue to consider is whether dose-response data from field studies and laboratory studies are consistent with each other. Figure 3-1 superimposes the data from the five studies described above. Although there is some variability in the dose-response sets, the data from all of the studies strongly overlap and are generally consistent with each other. This observation supports the conclusion that the laboratory-based dose-response curves are approximately applicable to site waters, and that if there are any effects of site water on toxicity, they are relatively small.

Figure 3-2 repeats the data shown in Figure 3-1, and adds other data from prior CDOW laboratory studies in non-acclimated fry. As seen, these historic data also overlap with and are consistent with the more recent field and laboratory studies. Based on this, the data set shown in Figure 3-2 was used to derive the final dose-response equation. The best fit parameters are as follows:

Response (% mortality) = 0.022 + 0.978·Φ[-1.564 + 1.462·ln(HQ)]

**4.0 COMPUTATIONAL TOOL**
Attachment C is an Excel spreadsheet that implements the calculation of HQ and the estimation of predicted excess mortality based on the equations presented above. This spreadsheet may be used as a tool to calculate the predicted increase in mortality in brown trout fry due to any specified level of zinc and hardness in the water. Some selected example results are shown in Figure 3-3.

5.0 SUMMARY

The results from on-site and laboratory studies of zinc toxicity in non-acclimated brown trout fry indicate that brown trout toxicity data from laboratory and field studies are generally consistent, and that mortality may be reasonably predicted from the combined data set using the following equation:

Response (% mortality) = 0.022 + 0.978 \cdot \Phi[-1.564 + 1.462 \cdot \ln(HQ)]

where:

HQ = \frac{Zn \text{ concentration}}{\exp[1.846 + 0.9634 \cdot \ln(H)]/2.5}

6.0 REFERENCES

