WATER POLLUTION STUDIES

Federal Aid Project #F-243R-6

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

Colorado Division of Wildlife

Fish Research Section

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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Division of Wildlife policy by the Director or Wildlife Commission.

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Title: Water Pollution Studies

Period Covered: July 1, 1999 to June 30, 2000

Principal Investigator: Patrick H. Davies

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Objective: To develop quantitative chemical and toxicological data on the toxicity of pollutants to aquatic life, investigate water pollution problems in the field, and provide expertise in aquatic chemistry and aquatic toxicology.

STUDY PLAN A: REGULATORY AND LEGAL ACTIVITIES

Objective: To provide technical assistance to regulatory and legal entities toward the development, implementation, and enforcement of water quality standards needed to protect or enhance the aquatic resources of Colorado.

Job A1. Water quality standards for the protection of aquatic life in Colorado

Job Objectives:

A1.1 To apply research results and toxicological information from literature toward the development, enactment, and implementation of water quality standards and appropriate aquatic life use classifications.

A1.2 To provide technical information and/or expert testimony in aquatic toxicology and aquatic chemistry in agency meetings, regulatory hearings, and/or court litigations as needed to protect aquatic resources of Colorado.

A1.3 To develop or compile toxicological and chemical data on toxicants for which state or federal governments have not developed a standard.

Work Schedule: Personnel will provide information and expertise in aquatic toxicology and chemistry in hearings before the Water Quality Control Commission, court litigations and as needed by other regulatory agencies. Consequently, a specific work schedule cannot be provided except as provided in Job A1.3a. Approach: A1.1 through A1.3.

ACCOMPLISHMENTS**:**

Approximately ten percent of Pat Davies time was spent assisting Vicky Peters, of the Colorado Attorney Generals Office, and State and Federal Trustee's on issues related to Natural Resources Damage (NRD) claims, restoration needs and goals, and settlements on the Arkansas River at Leadville, CO. These negotiations will continue.

Job A2. Water quality standards to sustain and protect trout in the Animas River below Silverton, CO.

Job Objective: To conduct insitu toxicity tests on brown, brook, and cutthroat trout in the Animas River for the purpose of developing site specific, acute and chronic water quality standards for zinc and possible other metals and provide information needed by engineers to determine the level of cleanup needed to meet downstream water quality standards.

A2.1 Compile and evaluate to determine seasonal water quality characteristics and metal concentrations in the Animas River below Silverton. Determine which metals (zinc, cadmium, and/or copper) need to be evaluated using insitu toxicity tests and at what seasonal hardness concentrations.

A2.2 Conduct insitu toxicity tests on brown and brook trout in June during spring run-off and again during the early spring at the initiation of spring run-off, end of April or early May,2000. Similar tests will be performed with cutthroat trout during October, 1999.

A2.3 Toxicity tests will determine 96 hr. LC50 concentrations for the metal(s) of concern and the lowest effect and no-effect concentrations occurring in a seven day period.

A2.4 Make recommendations for cleanup and target metals concentrations needed to attain self-sustaining trout population below Silverton.

ACCOMPLISHMENTS:

Toxicity of Copper and Zinc to Three Species of Trout in the Animas River and Laboratory Water

INTRODUCTION

A cooperative investigation between the Animas River Stakeholders, Colorado Water Quality Control Division and the Division of Wildlife was conducted on the Animas River below Silverton, CO. The purposes of this study were: 1) To determine the toxicity of different metals to trout in Animas River under water quality conditions reflecting seasonal variations, 2) Develop and recommend concentration targets for different metals of concern (i.e. remediation or clean-up goals) that will be needed to make the Animas River capable of sustaining a viable, self-reproducing trout population below Silverton.

An analysis of available metals data from the Animas River identified copper (Cu) and zinc (Zn) as the metals of primary concern relative to the survival of trout in the Animas River downstream of Silverton. The relative sensitivity of the trout species to Cu and Zn were investigated during seasonal *insitu* toxicity tests. Three seasons were selected to evaluate Cu and Zn toxicity to brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), and cutthroat trout (*Oncorhyncus clarki*). These were: Spring - high flow (June, 1999), Fall - low flow (October, 1999), and Early Spring - beginning of spring runoff (early April, 2000). However, Early Spring toxicity tests were delayed until May 1, 2000 due to replacement of railroad tracks removed during the filming of a movie during March and April. Toxicity tests on brown and brook trout were performed during both spring periods on the Animas River. *Insitu* toxicity tests on cutthroat trout were conducted only during the fall season. Similar toxicity test were also performed in DOW's Fort Collins, Aquatic Toxicology/Chemistry Laboratory under water quality conditions simulating those found seasonally in the Arkansas River.

METHODS AND MATERIALS

Animas River *Insitu* Toxicity Tests

Insitu toxicity test were conducted from a 32-ft travel trailer modified to support two flow through toxicity test systems, and house and support up to four research personnel. The trailer was positioned next to Durango-Silverton Narrow Gage Railroad (DSNGR) tracks adjacent to the Animas River at the mouth of the canyon about two miles downstream of Silverton, CO. Insulated 300 and 225 gallon polyethylene water tanks were positioned next to the trailer.

Each morning, DSNGR personnel would take us into the Animas River canyon on a pop-car pulling a flat car containing another 300 gallon water tank. Water was pumped from the Animas River down stream of Kendell Creek but upstream of Deer Creek. Water was transferred from the flat car into the insulated water tanks at the trailer. Water was pumped from the river reach below Kendell Creek because it represented an area of complete mixing of the Animas River with Mineral Creek, a steam heavily impacted by past mining activities.

<u>Water and Toxicant Delivery Systems</u>

A 12-volt submersible pump (Teel) delivered the stored water through a treatment system containing 600 g of Chelex 20 resin (Biorad products) prior to delivery to the toxicant diluter systems. The purpose of the Chelex resin treatment was to remove heavy metals that were present in the Animas river at levels deemed potentially toxic to the test organisms. Prior to use, the Chelex resin was thoroughly rinsed with well water located at the Fort Collins aquatic toxicology lab so as to put the resin in the calcium and magnesium form. The resin had no measurable effects on water quality parameters such as hardness, alkalinity, or pH. Treated Animas River water then supplied two modified Benoit continuous flow diluters housed in the trailer. One diluter was used to dispense Cu solutions to the test organisms, the other Zn. The diluters were constructed of teflon and vinyl tubing (FDA grade), polyethylene containers and silicone stoppers. Each diluter delivered 5 concentrations and a control using a 50% dilution series. During the spring of 1999, the diluters delivered 50 mls per minute to each of six, 6.5 liter polyethylene rubbermaid containers with lids. Larger fish tanks and diluter flows were needed to accommodate ASTM volume and flow requirements of the larger brown and brook trout used in these tests. During the fall of 1999, each diluter delivered 25 mls per minute to each of five duplicate concentrations and controls tanks. The volume of each of the twelve tanks containing ten cutthroat trout was three liters. For the tests conducted in early spring of 2000, test conditions were the same as the fall cutthroat trout tests except the one replicate tested brown trout while the other simultaneously tested brook trout. Ten fish were placed into each of the tanks during all of the Animas River Toxicity tests. A ½ inch hole in each fish tank was fitted with an elbow that enabled overflow of test water. Peristaltic pumps were used to deliver Cu and Zn toxicant solutions to the mixing chambers in each of the diluters. Copper and zinc sulfate salts with 5 and 7 waters of hydration, respectively, were used for making the test stock solutions.

During the *insitu* toxicity test in treated Animas River water, we also seasonally exposed brown, brook, or cutthroat trout to untreated Animas River water (i.e. ambient Animas River water prior to removal of metals by passing water through Chelex columns) to assess its potential acute and sub-chronic toxicity to these species.

<u>Acute and Chronic Toxicity Tests</u>

Acute toxicity data were collected during each of the tests from which 96-hr LC50 were estimated using the Trimmed Spearmann-Karber Technique. Data from each of the tests were collected for an additional period of time to provide an estimate of “chronic” mortality. Test durations range from five to nine days. Sub-chronic values, i.e. less than normal chronic exposure periods, were calculated as the geometric mean of the “lowest observed effect concentration” (LOEC) and the “no observed effect concentration” (NOEC). In some experiments, where substantial mortality occurred at the next higher concentration above that at which no mortallity occurred, it was assumed that given a longer chronic exposure period significant mortality would have occurred at the lower concentration level. The concentration was described as an “estimated effect” so as not to be confused with the LOEC designation. Similarly, where a NOEC could not be clearly defined, the term “estimated no-effect” concentration was used. LC50's and sub-chronic values were calculated using dissolved metal concentrations , when available. Lengths and weights were recorded on all fish that died during the experiments and upon termination of each of the tests.

Water quality characteristics, in accordance with Standard Methods, for hardness, alkalinity, pH, dissolved oxygen, temperature and conductivity were collected daily during each of the experiments. Water samples for metal analysis were also collected daily for total and dissolved metal analysis. Total metal water samples were collected in 2 oz Nalgene, high density polyethylene (HDPE) bottles acidified with 10 drops of triple distilled nitric acid (Ultrex) to pH <2. Dissolved metal water sample were similarly collected except a 60 ml sample was filtered through a 0.45 micron filter into a 60 ml HDPE bottle and acidified as above. Zinc concentrations were measured using Instrumental Laboratory Video 22 flame atomic absorption spectrophotometry

(AAS) with Smith-Hieftje background correction. Copper concentrations were determined with Thermo Jarrell Ash SH4000 atomic absorption spectrophotometer with CTF 180 graphite furnace using Smith-Hieftje background correction. Control samples from each of the experiments and pre-treatment Animas River samples were analyze for total and dissolved concentrations of eleven different elements using a Thermo Jarrell Ash, inductively coupled plasma (ICP) spectrometer, with a CETAC Ultrasonic Nebulizer, Model U-5000AT.

Organisms

Spring 1999: Brown trout eggs were collected from North Delaney Butte Reservoir in Colorado. Brook trout eggs were received from Dubois Hatchery in Wyoming

Fall 1999: Cutthroat eggs were collected from Trapper's Lake in Colorado and raised at the Colorado Division of Wildlife Glenwood Springs Fish Hatchery and transported to the Toxicology Laboratory post swimup.

Early spring 2000: Brown trout eggs were collected from North Delaney Butte Reservoir in Colorado. Brook Trout eggs were received from the Egan State Fish Hatchery in Utah and reared at the Aquatic Toxicology Laboratory in Fort Collins.

Laboratory Toxicity Tests

The laboratory Cu and Zn toxicity tests were conducted using both Benoit continuous flow and Mount and Brungs proportional diluters and procedures described above for the Animas River tests. The sources of fish were the same. The water source for the laboratory experiments was dechlorinated, Fort Collins tap water with a water hardness of about 50 mg/liter as calcium carbonate.

RESULTS

Animas River *Insitu* Acute Copper Toxicity

Very similar acute toxicity results in Animas River water were obtained on the toxicity of Cu to brown and brook trout during the spring of 1999 and early spring 2000 testing periods (Table 1; Tables 3 and 5, and Tables 9 and 11, respectively). Data from all acute toxicity tests are summarized in Table 1. Complete data from each of the tests are also cited in their respective tables). During the Early Spring 2000 tests, the three fold decrease in acute Cu toxicity, compared to the Spring of 1999, appears to be related to a greater sensitivity to the smaller brown and brook trout, where lengths and weights were 46 and 29 mm versus 67 and 74 mm, respectively (Table 1). However, with both colder water temperatures and higher water hardness, we would normally expect to see higher LC50 concentrations (i.e., less toxicity to Cu) in spring 2000 compared to the spring of 1999 (Table 1). The lower LC50 concentrations, of 9.8 and 11.9 for brown and brook trout, respectively, do not appear reasonable and cannot be explained. We see no water quality factors (Table 31) or a presence of other metals interactions (Table 26) that would account for this increase in toxicity. We also find no other data on brown and brook trout from experiments in our laboratory or the literature that would support these results. Therefore, we believe these data to be suspect and should probably be considered an anomaly.

During the Fall of 1999 cutthroat trout test in Animas River water, at a colder water temperature of 6.9°C and a much higher water hardness of 210 mg/liter, we see a significantly decreased level of Cu toxicity compared to the Spring of 1999 test on brown and brook trout. (Table 1; and Table 7). These data would suggest that the substantially higher level of hardness was responsible for the decreased level in acute toxicity to cutthroat trout, generally shown to be a more sensitive trout species compared to brown and brook trout.

Laboratory Acute Copper Toxicity

The apparent effect of reduced toxicity with increased hardness seen in the Animas River, Fall 1999, Cu test with cutthroat trout, is not evident in the laboratory toxicity test with cutthroat trout. In this laboratory test also during the Fall of 1999, the 96-hr LC50 for Cu was 47.8 µg/liter compared to 43.9 µg/liter obtained from the Animas River. The LC50s were essentially the same despite having a water hardness one-forth that occurring during the Animas River test (Table 1; and Tables 17 and 7, respectively). This apparent discrepancy, i.e. similar LC50s at very different level of water hardness, is most likely the consequence of using larger cutthroat during the laboratory test (94 mm and 7.03 g) versus 34 mm and 0.24 g fish for the Animas River test (Table 34). Decreased water temperature appears to be an important factor in decreasing the toxicity of metals. Comparing acute toxicity values obtained in laboratory water with cutthroat trout of similar size and similar water hardness, we see a two-fold reduction in LC50 concentrations. During the Early Spring 2000 toxicity test, a 96-hr LC50 of 23.6 µg Cu/liter was determined at a water temperature of 12°C compared to an LC50 of 47.8 µg Cu/liter at a water temperature of 6.5 °C during the fall 1999 testing period (Table 1; and Table 17 and 23, respectively). Of the three species tested in the laboratory, cutthroat trout (Early Spring 2000) were clearly more sensitive than brown and book trout (Spring 1999) to Cu at water temperature of 12 °C and similar water hardness (Table 1; and Tables 13, 15, and 23). As with the Animas River tests, 96-hr LC50 concentrations for brown and brook trout were similar during the spring 1999 and 2000 test periods (Table 1; Tables 13 and 15, and Tables 19 and 20, respectively).

Animas River and Laboratory Acute Zinc Toxicity

We do not see the similarity in 96-hr LC50 values in the toxicity of Zn to brown and brook trout as was found with Cu. Zinc is clearly more toxic to brown trout than to brook trout in both Animas River and laboratory waters (Table 1; Tables 4 and 6, Tables 14 and 16, and Tables 21 and 22). As seen in the spring 1999 (brown and brook trout) and early spring 2000 (cutthroat trout) laboratory toxicity tests at the same water temperature and similar water hardness, cutthroat trout were considerably more sensitive to the toxicity of Zn than brown trout and least sensitive brook trout (Table 1; Table 24, and Tables 14 and 16, respectively). Water hardness also plays a much more important role in reducing the toxicity of Zn than it does for Cu. A greater than four fold increase in the LC 50 concentrations comparing laboratory toxicity results of cutthroat at a hardness of 51 mg/liter with results measured in the Animas River at a hardness of 210 mg/liter during the fall 1999 experiments (Table 1; Tables 8 and 18). The 96 hr LC50 results obtained during the Early Spring, Animas River, Zn toxicity tests on the brown and brook trout are higher than expected compared to results obtained in the Spring of 1999. Two days into the experiment, we noticed that the Zn toxicant delivery tube to the diluter was partially pinched in a cabinet door. Later, upon running Zn analysis in the laboratory, we found that Zn concentrations in the fish tanks were reduced by two-thirds levels found on the 1st and 3rd days of the experiment. This reduced the mortality response and probably resulted in an increased tolerance that altered the toxicity results of the experiment. Consequently, data from these two tests are not valid.

Animas River *Insitu* and Laboratory Sub-Chronic Toxicity of Copper and Zinc

Sub-Chronic toxicity relationships of Cu and Zn to brown, brook, and cutthroat trout in Arkansas River and laboratory waters are the same as those described in the acute toxicity sections above and are not reiterated here. Sub-chronic results were determined by extending exposure time beyond the 96 hr acute time frame (Table 2). Copper and Zn mortality effects at the lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) were evaluated over an additional 1 to 4 days. Despite the fact that a 5 to 9 day exposure period constitutes a rather short period of time in which to define chronic toxicity levels, it does provide a sufficient period of time for tests conducted in moderately soft water from which chronic mortality levels can be estimated. For these reasons, where LOEC and NOEC levels could not be clearly defined, we took a conservative position in estimating the Sub-Chronic Values reported (Table 2). Exposure concentrations and mortality results for each of the experiments summarized in Table 2 are reported for each of the experiments conducted on the Animas River and laboratory (Table 3 through Table 24). Sub-Chronic Values were calculated as a geometric mean of the LOEC (or “estimated effect”)and NOEC (or estimated “no-effect”) values selected (shown in italics), and constitute a value presumed to be safe under chronic exposure for the species involved.

Acute and Sub-Chronic Toxicity of Untreated Animas River Water

For the most part, untreated Animas River water was not acutely and sub-chronically toxic to either brown or brook trout during the 1999 and 2000, spring toxicity experiments. At both times, untreated Animas River dissolved Cu and Zn concentrations were less than levels needed to cause either acute or sub-chronic mortality (Table 25 and Tables 1 and 2).

During the Fall of 1999, untreated Animas River water toxicity tests, 20 percent of the cutthroat trout died in 96 hours, with 35 percent mortality in 5 days of sub-chronic exposure (Table 25). The fall was also the season of highest metal concentrations (Table 26). However, it is also the season of highest water hardness, i.e. 212 mg/liter (Table 24). Cutthroat are the more sensitive of the three species tested as shown in the early spring 2000 laboratory experiments with Cu and Zn (Tables 1 and 2; Tables 23 and 24). We do not have toxicity data on cutthroat trout from laboratory experiments at a comparable level of water hardness (i.e., 210 mg/liter) found in the Animas River experiments. However, EPA’s, hardness based equations for determining acute and chronic criteria for Cu and Zn, can be used to indicate potential toxicity for a sensitive species like cutthroat trout where concentrations in untreated Animas River water exceed criteria limits. Total and dissolved metal concentrations, as measured by ICP, are reported for the Animas River pre-treated and post-treatment experiment controls (Table 26). Based on seasonal hardness data and dissolved concentration of Cu, Zn, and Cd reported in Table 25, it would appear, the acute and sub-chronic toxicity found with cutthroat trout at a water hardness of 212 mg/liter during the fall of 1999 was caused by exceeding a factor of two chronic criteria limits for Zn and cadmium (Table 27). Chronic criteria limits of Zn and Cd are 199 and 2.03, respectively, compared to exposure of cutthroat trout to dissolved Zn and Cd concentrations of 545 and 4.04 µg/liter, respectively. ICP metal analysis of dechlorinated Fort Collins city water used in the laboratory experiments is also provided (Table 28).

Water Quality Characteristic and Trout Lengths and Weights

Seasonal Animas River and laboratory water quality characteristics for each of the experiments are reported. Chelex treatment essentially had no effect on hardness, alkalinity, pH, temperature, dissolved oxygen, and conductivity (Tables 29 through 31). However, following the analysis of metal samples collected daily, we learned that the two 600 g Chelex columns were not adequate to bind all the metals present in the Animas River. After a period of about 7 days, metal concentrations started to pass through the columns, as seen by the range of metal concentrations reported for the Chelex treated control waters (Table 26). Water quality characteristics for the laboratory source water are also reported (Tables 32 and 33). Fish lengths and weights, during both the Animas River and laboratory studies, varied seasonally depending on the age of each of the species at the time the tests were performed (Table 34).

DISCUSSION AND CONCLUSIONS

Based on Animas River and laboratory toxicity data, brown and brook trout are equally sensitive to the toxicity of copper (Cu). Brook trout generally appear to be approximately three times more tolerant to the toxicity of Zn than brown trout. However, during the early spring of 2000 laboratory toxicity tests, both species appeared to be equally sensitive to Zn. (Tables 1 and 2; Tables 22 and 24). Difference in sensitivity may have been masked because of control mortality that occurred with both species under both field and laboratory conditions. Cutthroat trout, under similar water quality and temperature conditions, are more sensitive of the three species tested for both Cu and Zn (Tables 1 and 2; Tables 13,15, and 23 and Tables 14,16, and 24, respectively).

Copper appears to be a metal of concern relative to the potential toxic affects on the Animas River. Dissolved copper concentrations in the Animas River are near levels found to be chronically toxic to brown and brook trout and generally exceed EPA acute and chronic criteria (Table 26, Tables 1 and 2, and Table 27).

Zinc is also a metal of concern but probably less so than Cu. Dissolved Zn concentrations in the Animas River (even though they exceed acute and chronic criteria - Table 27) are generally less than levels found to be acutely or chronically toxic to either brown or brook trout (Table 26; and Table 1). Total metal concentrations for other toxic metals are high for: aluminum, cadmium, iron, manganese, and lead. However, dissolved concentrations of these metals are generally below toxic levels, except for cadmium (Cd). Dissolved Cd concentrations frequently approach or exceed potentially toxic levels for brown, brook and cutthroat trout. However, without site toxicity studies on Cd, its potential toxicity in Animas River water can only surmised. If we relate dissolved Cd concentrations in the Animas River to EPA's hardness based criteria, Cd might be another metal of concern, at least in relation to EPA's criteria (compare Table 25 and 26 with Table 27). Acute and chronic tests in our laboratory on brown trout in water of different hardness suggest dissolved Cd levels in the Animas River would be toxic to brown and brook trout.

As mentioned above, where mortality did not provide a clear distinction between LOEC and NOEC concentrations, we took a conservative position in estimating Sub-chronic "effect" and "no-effect" toxicity levels. It must be remembered from the methods section, that we pumped Animas River water from down in the canyon each morning and this water characterized the river for the 24 hour period during which we ran toxicity tests. This provided a very small window of only about 5 minutes to reflect water quality characteristic of the river. Also, during the spring testing periods, we frequently observed high flows and much higher sediment load in the river during the afternoon when air temperatures had increased. It is likely that we would also find higher metal concentrations than measured in water collected in the early morning.   With the 5 to 9 day sub-chronic test durations, mortality was the only effect that could be measured. Generally, reduced growth is a more sensitive measure of adverse chronic effects. Consequently, we were unable to assess a more sensitive indicator of harm. We also tested the toxicity of Cu and Zn separately, and because of the treatment of Animas River water (Chelex columns) we removed other metals that could possibly increased toxicity through additive or synergistic interactions.

RECOMMENDATIONS

We recommend attempts be made to develop water quality conditions capable of supporting a brown and/or brook trout fishery below Silverton. Because of its higher sensitivity to metals cutthroat trout are not recommended for the Animas River below Silverton. To attain a viable fishery, cleanup of point and non-point sources of metals in the Animas River basin at Silverton should achieve the following target levels of Cu and Zn based on the Animas River toxicity tests. These levels need to be meet during spring runoff, the period of lowest water hardness and greatest vulnerability of brown and brook trout.

Copper levels needed to support brown or brook trout in the Animas River:
Brown or Brook Trout: 20µg/liter

Zinc levels needed to support brown or brook trout in the Animas River:
Brown Trout: 250 µg/liter or less
Brook Trout: 700 µg/liter or less

Cadmium levels needed to support brown or brook trout in the Animas River, were not tested in Animas River water, based on laboratory and literature values :
Brown or Brook Trout: 1.2 µg/liter

Table 1. Comparison of seasonal **96 hr LC50 concentrations** for brown, brook, and cutthroat trout from insitu acute toxicity tests in the Animas River with results determined in the laboratory using similar water quality. Temperature (°C), water hardness (mg/l), and length (mm) are provided for comparison. Ninety-five percent confidence interval in parentheses

ANIMAS RIVER - 96 Hr LC50 (µg/L)					LABORATORY - 96 Hr LC50 (µg/L)				
LC50 (95%C.I.)	Species	°C	mg/l	mm	LC50 (95%C.I.)	Species	°C	mg/l	mm
COPPER - Spring 1999									
30.1 (26.6-34.1)	Brown	11.7	42.3	67	33.9 (29.5-38.9)	Brown	12.0	52.6	92
31.9 (26 - 39)	Brook	11.3	45.0	74	48.2 (42.5-54.8)	Brook	12.0	52.6	119
COPPER - Fall 1999									
43.9 (39.4-48.9)	Cutthroat	6.9	210	34	47.8 (40.1-57.1)	Cutthroat	6.5	50.4	94
COPPER - Early Spring 2000									
9.8 (8.1-11.7)	Brown	8.3	81.3	46	57.3 (49.4-66.4)	Brown	10.8	53.3	63
11.9 (9.5-14.9)	Brook	8.3	81.3	29	74.6 (65.3-85.2)	Brook	10.8	53.3	42
					23.6 (19.2-29.0)	Cutthroat	12.0	41.2	76
ZINC - Spring 1999									
476 (375-603)	Brown	11.7	42.3	67	484 (340-689)	Brown	12.0	52.6	92
1526(1221-1906)	Brook	11.3	45.0	74	1178 (981-1414)	Brook	12.0	52.6	119
ZINC - Fall 1999									
1749(1575-1942)	Cutthroat	6.9	210	34	411 (250-677)	Cutthroat	6.5	51.3	92
ZINC - Early Spring 2000									
See Table 10	Brown	8.3	81.3	46	603 (391-931)	Brown	10.7	54.6	63
See Table 12	Brook	8.3	81.3	29	738 (612-891)	Brook	10.7	54.6	42
					130 (109-155)	Cutthroat	12.0	40.8	72

Table 2. Comparison of seasonally **estimated Sub-chronic Values** and exposure period (days) for brown, brook, and cutthroat trout from insitu tests in the Animas River with laboratory sub-chronic toxicity tests. Where possible, results are reported for dissolved copper and zinc. Except of Fall 1999 cutthroat trout experiments, laboratory tests were conducted using water quality characteristics similar to conditions occurring in the Animas River. A chronic value is calculated as a geometric mean of the "no observed effect concentration" (NOEC) and the "lowest observed effect concentration" (LOEC), in italics. Water quality conditions for each test are as reported in Table 1.

ANIMAS R.: Sub-Chronic Value (µg/L)					**LABORATORY: Sub-Chronic Value (µg/L)**				
Species	Days	LOEC	NOEC	C.V.	Species	Days	LOEC	NOEC	C.V.
COPPER - Spring 1999									
Brown	5	34.2	15.0	**22.6**	**Brown**	7	33.2	17.7	**24.2**
Brook	7	22.4	16.0	**18.9**	**Brook**	7	34.7	18.8	**25.5**
COPPER - Fall 1999									
Cutthroat	8	15.4	23.5	**19.0**	**Cutthroat**	7	27.2	14.0	**19.5**
COPPER - Early Spring 2000									
Brown	9	9.7	6.5	**7.9**	**Brown**	7	35.4	18.8	**25.8**
Brook	9	9.7	6.5	**7.9**	**Brook**	7	35.4	18.8	**25.8**
					Cutthroat	14	25.0	13.8	**19.2**
ZINC - Spring 1999									
Brown	7	411	232	**309**	**Brown**	7	320	171	**234**
Brook	7	1059	585	**787**	**Brook**	7	1103	608	**819**
ZINC - Fall 1999									
Cutthroat	8	737	441	**570**	**Cutthroat**	6	268	(150)	**200**
ZINC - Early Spring 2000									
Brown	9	1269	699	**Table 10**	**Brown**	7	484	221	**327**
Brook	9	1269	699	**Table 12**	**Brook**	7	484	221	**327**
					Cutthroat	10	116	51.3	**77.1**

Table 3. **Animas River** toxicity tests during the **Spring, 1999**: Average concentrations of **copper** (Cu) and associated mortality of (67 mm) **brown trout** after 96 hours and 5 days of exposure at water temperature (11.7°C) and hardness (42.3 mg/L) Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu Concentration (µg/L)	240	120	60	30	15	0
Total Cu Concentration (µg/L)	242.5 (14.8)	122.5 (0.7)	91.8 (33.5)	52.6 (9.6)	30.8 (4.8)	15.2 (3.8)
Dissolved Cu Concentration (µg/L)	**138.0** (-)	**86.0** (-)	**34.2** (3.3)	***23.2**** (3.6)	***15.0*** (4.1)	**5.6** (1.1)
96 Hour Mortality (%)	100	100	90	0	0	0
5 Day Mortality (%)	100	100	90	0	0	0

96 Hour LC50 = 30.1 µg Cu /L. 95% Confidence Interval = 26.6 - 34.1 µg Cu /L
Estimated Sub-Chronic Value= 18.6 µg Cu /L (assume NOEC to be 15 µg/L and significant mortality at an *estimated "effect concentration" of 23.2 µg/L with longterm chronic exposure).

Table 4. **Animas River** toxicity tests during the **Spring, 1999**: Average concentrations of **zinc** (Zn) and associated mortality of (67 mm) **brown trout** after 96 hours and 7 days of exposure at water temperature (11.7°C) and hardness (42.3 mg/L) Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Zn Concentration (µg/L)	2400	1200	600	300	150	0
Total Zn Concentration (µg/L)	2220 (-)	1358 (25.4)	718 (81.9)	446 (78.0)	280 (58.0)	47.6 (23.0)
Dissolved Zn Concentration (µg/L)	**2146** (-)	**1281** (-)	**649** (99.8)	***411*** (95.1)	***232*** (34.7)	**22.4** (9.8)
96 Hour Mortality (%)	100	100	60	60	0	0
7 Day Mortality (%)	100	100	60	60	0	0

96 Hour LC50 = 476 µg Zn /L. 95% Confidence Interval = 375-603 µg Zn /L
Estimated Sub-Chronic Value= 309 µg Zn /L.

Table 5. **Animas River** toxicity tests during the **Spring, 1999**: Average concentrations of **copper** (Cu) and associated mortality of (74 mm) **brook trout** after 96 hours and 7 days of exposure at water temperature (11.3°C) and hardness (45.0 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu Concentration (µg/L)	240	120	60	30	15	0
Total Cu Concentration (µg/L)	234.5 (7.8)	123.7 (1.5)	85.8 (10.1)	56.4 (13.2)	30.1 (4.8)	13.5 (2.5)
Dissolved Cu Concentration (µg/L)	**174.0** (-)	**81.0** (7.1)	**42.7** (5.3)	***22.4*** (3.9)	***16.0*** (1.5)	**5.3** (0.4)
96 Hour Mortality (%)	100	100	70	10	0	0
7 Day Mortality (%)	100	100	80	20	0	0

96 Hour LC50 = 31.9 µg Cu /L. 95% Confidence Interval = 26-39 µg Cu /L.
Estimated Sub-Chronic Value = 18.9 µg Cu /L.

Table 6. **Animas River** toxicity tests during the **Spring, 1999**: Average concentrations of **zinc** (Zn) and associated mortality of (74 mm) **brook trout** after 96 hours and 7 days of exposure at water temperature (11.3°C) and hardness (45.0 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Zn Concentration (µg/L)	3000	1500	750	375	188	0
Total Zn Concentration (µg/L)	3284 (603)	1798 (192)	1106 (137)	608 (99.0)	456 (88.6)	120 (69.4)
Dissolved Zn Concentration (µg/L)	**3272** (622)	**1741** (216)	***1059*** (131)	***585*** (103)	**424** (94.8)	**99.7** (70.2)
96 Hour Mortality (%)	100	50	20	0	0	0
7 Day Mortality (%)	100	60	20	0	0	0

96 Hour LC50 = 1526 µg Zn /L. 95% Confidence Interval = 1221-1906 µg Zn /L.
Estimated Sub-Chronic Value= 787 µg Zn /L.

Table 7. **Animas River** toxicity tests during the **Fall, 1999**: Average concentrations of **copper** (Cu) and associated mortality of (34 mm) **cutthroat trout** after 96 hours and 8 days of exposure at water temperature (6.9°C) and hardness (210 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu Concentration (µg/L)	180	90	45	22.5	11.2	0
Total Cu Concentration (µg/L)	200 (13.0)	110 (6.8)	81.8 (7.5)	54.4 (3.6)	40.8 (2.8)	28.2 (4.8)
Dissolved Cu Concentration (µg/L)	**112** (9.6)	**58.3** (11.0)	**41.0** (10.9)	***23.5*** (6.2)	***15.4*** (3.5)	**6.4** (2.4)
96 Hour Mortality (%)	100 (0)	95 (7.1)	30 (14.1)	10 (0)	0 (0)	0 (0)
8 Day Mortality (%)	100 (0)	100 (0)	95 (7.1)	30 (0)	0 (0)	0 (0)

96 Hour LC50 = 43.9 µg Cu /L. 95% Confidence Interval = 39.4 - 48.9 µg Cu /L
Estimated Sub-Chronic Value= 19.0 µg Cu /L.

Table 8. **Animas River** toxicity tests during the **Fall, 1999**: Average concentrations of **zinc** (Zn) and associated mortality of (34 mm) **cutthroat trout** after 96 hours and 8 days of exposure at water temperature (6.9°C) and hardness (210 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics*.

Nominal Zn Concentration (µg/L)	2000	1000	500	250	125	0
Total Zn Concentration (µg/L)	2272 (123)	1435 (169)	778 (84)	475 (135)	372 (144)	193 (67)
Dissolved Zn Concentration (µg/L)	**2128** (210)	**1382** (171)	***737**** (89)	***441*** (139)	**340** (147)	**169** (75)
96 Hour Mortality (%)	75 (7.1)	20 (14.1)	0 (0)	0 (0)	0 (0)	0 (0)
8 Day Mortality (%)	80 (0)	50 (28.3)	5 (7.1)	0 (0)	0 (0)	0 (0)

96 Hour LC50 = 1749 µg Zn /L. 95% Confidence Interval = 1575 - 1942 µg Zn /L.
Estimated Sub-Chronic Value= 570 µg Zn /L (assume NOEC to be 441 µg/L and significant mortality at an *estimated "effect concentration" of 737 µg/L with longterm chronic exposure).

Table 9. **Animas River** toxicity tests during the **Early Spring, 2000:** Average concentrations of **copper** (Cu) and associated mortality of (46 mm) **brown trout** after 96 hours and 9 days of exposure at water temperature (8.3°C) and hardness (81.3 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu Concentration (µg/L)	25	12.5	6.25	3.12	1.56	0
Total Cu Concentration (µg/L)	71.8 (11.6)	41.0 (5.4)	28.4 (4.3)	26.0 (8.9)	19.3 (2.5)	16.7 (1.9)
Dissolved Cu Concentration (µg/L)	**31.0** (2.6)	**16.4** (2.4)	***9.7*** (1.3)	***6.5*** (3.3)	**6.0** (2.0)	**5.0** (0.6)
96 Hour Mortality (%)	100	100	50	10	10	10
9 Day Mortality (%)	100	100	50	10[1]	10[1]	20

[1] Concentrations added after start of test - Mortality after 6 days.

96 Hour LC50 = 9.8 µg Cu /L. 95% Confidence Interval = 8.1 - 11.7 µg Cu /L
Estimated Sub-Chronic Value= 7.9 µg Cu /L.

Table 10. **Animas River** toxicity tests during the **Early Spring, 2000:** Average concentrations of **zinc** (Zn) and associated mortality of (46 mm) **brown trout** after 96 hours and 9 days of exposure at water temperature (8.3°C) and hardness (81.3 mg/L). Standard deviations in parentheses.

Nominal Zn Concentration (µg/L)	5600	2800	1400	700	350	0
Total Zn Concentration (µg/L)	5333 (1566)	2467 (779)	1320 (393)	743 (231)	424 (125)	107 (66)
Dissolved Zn Concentration (µg/L)	**5228** (1576)	**2420** (784)	**1269** (389)	**699** (230)	**387** (127)	**85** (66)
96 Hour Mortality (%)	100	50	10	0	10	0
9 Day Mortality (%)	100	50	10	0	20	10

96 Hour LC50 = (2161??) µg Zn /L. 95% Confidence Interval = 1596 - 2925 µg Zn /L. **Because toxicant failure 2nd day, acute and sub-chronic test results compromised**.
Estimated Sub-Chronic Value

Table 11. **Animas River** toxicity tests during the **Early Spring, 2000:** Average concentrations of **copper** (Cu) and associated mortality of (29 mm) **brook trout** after 96 hours and 9 days of exposure at water temperature (8.3°C) and hardness (81.3 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu Concentration (µg/L)	25	12.5	6.25	3.12	1.56	0
Total Cu Concentration (µg/L)	71.8 (11.6)	41.0 (5.4)	28.4 (4.3)	26.0 (8.9)	19.3 (2.5)	16.7 (1.9)
Dissolved Cu Concentration (µg/L)	**31.0** (2.6)	**16.4** (2.4)	***9.7*** (1.3)	***6.5*** (3.3)	**6.0** (2.0)	**5.0** (0.6)
96 Hour Mortality (%)	100	70	50	0	0	0
9 Day Mortality (%)	100	70	50	0 [1]	0 [1]	0

[1] Concentrations added after start of test - Mortality after 6 days.

96 Hour LC50 = 11.9 µg Cu /L. 95% Confidence Interval = 9.5 - 14.9 µg Cu /L
Estimated Sub-Chronic Value= 7.9 µg Cu /L.

Table 12. **Animas River** toxicity tests during the **Early Spring, 2000:** Average concentrations of **zinc** (Zn) and associated mortality of (29 mm) **brook trout** after 96 hours and 9 days of exposure at water temperature (8.3°C) and hardness (81.3 mg/L). Standard deviations in parentheses.

Nominal Zn Concentration (µg/L)	5600	2800	1400	700	350	0
Total Zn Concentration (µg/L)	5333 (1566)	2467 (779)	1320 (393)	743 (231)	424 (125)	107 (66)
Dissolved Zn Concentration (µg/L)	**5228** (1576)	**2420** (784)	**1269** (389)	**699** (230)	**387** (127)	**85** (66)
96 Hour Mortality (%)	90	20	30	18	0	0
9 Day Mortality (%)	100	70	40	18	0	20

96 Hour LC50 = (2622??) µg Zn /L. 95% Confidence Interval = 1805 - 3808 µg Zn /L
Because toxicant failure 2nd day, acute and sub-chronic test results compromised.
Estimated Sub-Chronic Value

Table 13. **Laboratory** toxicity tests during the **Spring 1999**, average concentrations of **copper** (Cu) and associated mortality of (92 mm) **brown trout** after 96 hours and 7 days of exposure at water temperature (12.0°C) and hardness (52.6 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu (µg/L)	200	150	112	72	36	20	0
Total Cu (µg/L)	213 (1.4)	149 (1.4)	111 (5.7)	66.0 (4.2)	38.8 (5.3)	21.5 (3.2)	<10 (0.6)
Dissolved Cu (µg/L)	**204** (4.9)	**137** (4.2)	**96** (1.4)	**54.5** (6.0)	***33.2*** (3.2)	***17.7*** (4.8)	**<10** (1.7)
96 Hour Mortality (%)	100	100	100	100	40	0	0
7 Day Mortality (%)	100	100	100	100	47	0	0

96 Hour LC50 = 33.9 µg Cu /L. 95% Confidence Interval = 29.5 - 38.9 µg Cu /L
Estimated Sub-Chronic Value = 24.2 µg Cu /L.

Table 14. **Laboratory** toxicity tests during the **Spring 1999**, average concentrations of **zinc** (Zn) and associated mortality of (92 mm) **brown trout** after 96 hours and 7 days of exposure at water temperature (12.0°C) and hardness (52.6 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Zn (µg/L)	2000	1500	1125	720	360	200	0
Total Zn (µg/L)	2006 (57)	1510 (140)	1115 (30)	627 (16)	335 (6)	178 (8)	<10 (4.8)
Dissolved Zn (µg/L)	**1990** (42)	**1467** (141)	**1066** (44)	**589** (19)	***320*** (8)	***171*** (8)	**<10** (3.8)
96 Hour Mortality (%)	100	100	80	47	27	7	0
7 Day Mortality (%)	100	100	87	53	33	7	0

96 Hour LC50 = 484 µg Zn /L. 95% Confidence Interval = 340 - 689 µg Zn /L
Estimated Sub-Chronic Value = 234 µg Zn /L (Assume mortality effect at 171 µg/L is not significant, and estimate the "no-effect" concentration to be 171 µg/L, and a LOEC of 320 µg/L.

Table 15. **Laboratory** toxicity tests during the **Spring 1999**, average concentrations of **copper** (Cu) and associated mortality of (119 mm) **brook trout** after 96 hours and 7 days of exposure at water temperature (12.0°C) and hardness (52.6 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu (µg/L)	240	180	135	86.4	43.2	24.0	0
Total Cu (µg/L)	241 (0.7)	172 (2.1)	132 (0.0)	74.2 (5.6)	40.2 (2.7)	22.5 (3.6)	<10 (3.2)
Dissolved Cu (µg/L)	**222** (0.0)	**162** (3.5)	**110** (2.1)	**62.3** (4.3)	***34.7*** (2.4)	***18.8*** (1.9)	**<10** (1.8)
96 Hour Mortality (%)	100	100	100	87	7	0	0
7 Day Mortality (%)	100	100	100	100	13	0	0

96 Hour LC50 = 48.2 µg Cu /L. 95% Confidence Interval = 42.5 - 54.8 µg Cu /L
Estimated Sub-Chronic Value = 25.5 µg Cu /L.

Table 16. **Laboratory** toxicity tests during the **Spring 1999**, average concentrations of **zinc** (Zn) and associated mortality of (119 mm) **brook trout** after 96 hours and 7 days of exposure at water temperature (12.0°C) and hardness (52.6 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Zn (µg/L)	2000	1500	1125	720	360	200	0
Total Zn (µg/L)	1994 -	1574 (36)	1138 (49)	636 (15)	332 (8)	202 (1)	<10 (1.4)
Dissolved Zn (µg/L)	**1960** -	**1553** (45)	***1103*** (24)	***608*** (5)	**324** (0)	**172** (4)	**<10** (2.8)
96 Hour Mortality (%)	100	100	7	0	0	7	0
7 Day Mortality (%)	100	100	7	0	0	7	0

96 Hour LC50 = 1178 µg Zn /L. 95% Confidence Interval = 981 - 1414 µg Zn /L
Estimated Sub-Chronic Value = 819 µg Zn /L ((assume NOEC to be 608 µg/L and significant mortality at an *estimated "effect concentration" of 1103 µg/L with longterm chronic exposure).

Table 17. **Laboratory** toxicity tests during the **Fall 1999**, Average concentrations of **copper** (Cu) and associated mortality of (94 mm) **cutthroat trout** after 96 hours and 7 days of exposure at water temperature (6.5°C) and hardness (50.4 mg/L). Standard deviations in parentheses.

Nominal Cu (µg/L)	150	112	84	48	27	15	0.0
Total Cu (µg/L)	151 (1.73)	107 (5.51)	83.5 (0.71)	48.0 (1.41)	27.2 (1.10)	14.0 (1.10)	1.20 (0.84)
96 Hour Mortality (%)	100	100	100	50	0	0	0
7 Day Mortality (%)	100	100	100	50	0	0	0

96 Hour LC50 = 47.8 µg Cu /L. 95% Confidence Interval = 40.1 - 57.1 µg Cu /L
Estimated Sub-Chronic Value = 19.5 µg Cu /L (Assume NOEC of 27.2 is questionable and would have significant mortality at an *estimated "effect concentration" of 27.2 µg/L with longterm chronic exposure. Estimate a NOEC of 14.0).

Table 18. **Laboratory** toxicity tests during the **Fall 1999**, average concentrations of **zinc** (Zn) and associated mortality of (92 mm) **cutthroat trout** after 96 hours and 6 days of exposure at water temperature (6.5°C) and hardness (51.3 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Zn (µg/L)	3000	2250	1680	960	540	300	0.0
Total Zn (µg/L)	**2952** (150)	**2213** (112)	**1589** (45)	**902** (20.5)	**496** (14.8)	***268*** (6.8)	**3.75** (3.59)
96 Hour Mortality (%)	100	100	100	100	40	10	0
6 Day Mortality (%)	100	100	100	100	50	20	0

96 Hour LC50 = 411 µg Zn /L. 95% Confidence Interval = 250 - 677 µg Zn /L
Estimated Sub-Chronic Value = 200 µg Zn /L (Assumes: LOEC = 268 and estimate "no-effect" concentration at one-half, i.e.150 µg /L, the nominal effect concentration of 300)

Table 19. **Laboratory** toxicity tests during the **Early Spring 2000**, average concentrations of **copper** (Cu) and associated mortality of (63 mm) **brown trout** after 96 hours and 7 days of exposure at water temperature (10.8°C) and hardness (53.3 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

	1	2	3	4	5		C
Nominal Cu (µg/L)	140	70	35	17.5	8.8		0.0
Total Cu (µg/L)	134 (14)	74.9 (9.8)	45.6 (9.2)	21.4 (3.7)	10.8 (2.6)		1.36 (0.28)
Dissolved Cu (µg/L)	**124** (4)	**65.4** (4.6)	***35.4*** (2.9)	***18.8*** (2.4)	**8.6** (1.13)		**0.86** (0.42)
96 Hr Ave. Mort (%)	**95**	**70**	**5**	**0**	**0**		**0**
% Mortality - rep a	100	70	10	0	0		0
% Mortality - rep b	90	70	0	0	0		0
7 Day Ave. Mort (%)	**95**	**80**	**10**	**0**	**0**		**0**
% Mortality - rep a	100	80	10	0	0		0
% Mortality - rep b	90	80	10	0	0		0

96 Hour LC50 = 57.3 µg Cu /L. 95% Confidence Interval = 49.4 - 66.4 µg Cu/L
Estimated Sub-Chronic Value = 25.8 µg Cu /L.

Table 20. **Laboratory** toxicity tests during the **Early Spring 2000**, average concentrations of **copper** (Cu) and associated mortality of (42 mm) **brook trout** after 96 hours and 7 days of exposure at water temperature (10.8°C) and hardness (53.3 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

	1	2	3	4	5		C
Nominal Cu (µg/L)	140	70	35	17.5	8.8		0.0
Total Cu (µg/L)	134 (14)	74.9 (9.8)	45.6 (9.2)	21.4 (3.7)	10.8 (2.6)		1.36 (0.28)
Dissolved Cu (µg/L)	**124** (4)	**65.4** (4.6)	***35.4*** (2.9)	***18.8*** (2.4)	**8.6** (1.13)		**0.86** (0.42)
96 Hr Ave. Mort (%)	**100**	**25**	**5**	**0**	**0**		**0**
% Mortality - rep a	100	20	10	0	0		0
% Mortality - rep b	100	30	0	0	0		0
7 Day Ave. Mort (%)	**95**	**30**	**10**	**0**	**0**		**0**
% Mortality - rep a	100	30	10	0	0		0
% Mortality - rep b	100	30	10	0	0		0

96 Hour LC50 = 74.6 µg Cu /L. 95% Confidence Interval = 65.3 - 85.2 µg Cu/L
Estimated Sub-Chronic Value = 25.8 µg Cu /L.

Table 21. **Laboratory** toxicity tests during the **Early Spring 2000**, average concentrations of **zinc** (Zn) and associated mortality of (63 mm) **brown trout** after 96 hours and 7 days of exposure at water temperature (10.7°C) and hardness (54.6 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

	1	2	3	4	5		C
Nominal Zn (µg/L)	4000	2000	1000	500	250		0.0
Total Zn (µg/L)	4085 (191)	2145 (82)	1107 (72)	510 (28)	235 (36)		6.6 (5.1)
Dissolved Zn (µg/L)	**4070** (212)	**2099** (127)	**1047** (49)	***484*** (38)	***221*** (34)		**7.0** (5.9)
96 Hr Ave. Mort (%)	**100**	**100**	**45**	**60**	**20**		**25**
% Mortality - rep a	100	100	10	90	30		30
% Mortality - rep b	100	100	80	30	10		20
7 Day Ave. Mort (%)	**100**	**100**	**65**	**65**	**25**		**30**
% Mortality - rep a	100	100	30	90	40		40
% Mortality - rep b	100	100	100	40	10		20

96 Hour LC50 = 603 µg Zn /L. 95% Confidence Interval = 391- 931 µg Zn/L
Estimated Sub-Chronic Value = 327 µg Zn /L.

Table 22. **Laboratory** toxicity tests during the **Early Spring 2000**, average concentrations of **zinc** (Zn) and associated mortality of (42 mm) **brook trout** after 96 hours and 7 days of exposure at water temperature (10.7°C) and hardness (54.6 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

	1	2	3	4	5		C
Nominal Zn (µg/L)	4000	2000	1000	500	250		0.0
Total Zn (µg/L)	4085 (191)	2145 (82)	1107 (72)	510 (28)	235 (36)		6.6 (5.1)
Dissolved Zn (µg/L)	**4070** (212)	**2099** (127)	**1047** (49)	***484*** (38)	***221*** (34)		**7.0** (5.9)
96 Hr Ave. Mort (%)	**100**	**100**	**90**	**10**	**0**		**10**
% Mortality - rep a	100	100	100	20	0		20
% Mortality - rep b	100	100	80	0	0		0
7 Day Ave. Mort (%)	**100**	**100**	**95**	**10**	**0**		**10**
% Mortality - rep a	100	100	100	20	0		20
% Mortality - rep b	100	100	90	0	0		0

96 Hour LC50 = 738 µg Zn /L. 95% Confidence Interval = 612 - 891 µg Zn/L
Estimated Sub-Chronic Value = 327 µg Zn /L.

Table 23. **Laboratory** toxicity tests during the **Early Spring 2000**, average concentrations of **copper** (Cu) and associated mortality of (76 mm) **cutthroat trout** after 96 hours and 14 days of exposure at water temperature (12.0°C) and hardness (41.2 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Cu (µg/L)	90	60	44.8	25.6	14.4	8.0	0.0
Total Cu (µg/L)	**93.0** (9.16)	**72.0** (2.98)	**51.0** (2.83)	***25.0*** (2.33)	***13.8*** (1.44)	**9.1** (1.15)	**1.06** (0.60)
96 Hour Mortality (%)	100	90	100	70	0	0	0
14 Day Mortality (%)	100	100	100	70	0	0	0

96 Hour LC50 = 23.6 µg Cu /L. 95% Confidence Interval = 19.2 - 29.0 µg Cu /L
Estimated Sub-Chronic Value = 19.2 µg Cu /L.

Table 24. **Laboratory** toxicity tests during the **Early Spring 2000**, average concentrations of **zinc** (Zn) and associated mortality of (72 mm) **cutthroat trout** after 96 hours and 10 days of exposure at water temperature (12.0°C) and hardness (40.8 mg/L). Standard deviations in parentheses. *LOEC* and *NOEC* in *italics.*

Nominal Zn (µg/L)	500	375	280	160	90	50	0.0
Total Zn (µg/L)	**520** (19.1)	**400** (21.4)	**295** (15.0)	**168** (11.6)	***116*** (9.3)	***51.3*** (10.8)	**7.5** (6.30)
96 Hour Mortality (%)	100	100	100	90	20	0	0
10 Day Mortality (%)	100	100	100	90	20	0	0

96 Hour LC50 = 130 µg Zn/L. 95% Confidence Interval = 109 - 155 µg Zn/L
Estimated Sub-Chronic Value = 77.1 µg Zn/L.

Table 25. **Animas River** seasonal toxicity tests in **Untreated water:** Average concentrations of **copper** (Cu), **zinc** (Zn), and cadmium (Cd) and associated trout mortality in **ambient, untreated, Animas River water** after 96 hr and sub-chronic exposure. Standard deviations in parentheses and range in brackets..

Ambient Animas Water	**Spring 1999**	**Fall 1999**	**Early Spring 2000**
Hardness (mg/L)	44.5 (2.2) [43.7-46.0]	212 (8.7) [194-219]	83.6 (3.9) [76.8-86.4]
Temperature °C	11.2 (2.2) [8.6-13.6]	3.2 (1.9) [0.1-5.0]	7.4 (1.4) [5.4-8.5]
Total Cu (µg/L)	25.7 (9.7) [18-48]	49.3 (7.3) [44.4-66.3]	35.8 (17.0) [27.6-80.6]
Dissolved Cu (µg/L)	7.2 (2.9) [5-13]	12.0 (3.6) [9.1-20.3]	10.1 (1.4) [8.6-13.3]
Total Zn (µg/L)	245 (37.2) [207-328]	643 (10.3) [624-656]	370 (43.4) [334-476]
Dissolved Zn (µg/L)	171 (11.7) [160-195]	545 (174) [120-633]	321 (24.7) [286-362]
Total Cd (µg/L)	1.0 (0)	4.65 (0.11) [4.5-4.8]	1.56 (0.14) [1.39-1.74]
Dissolved Cd (µg/L)	1.0 (0)	4.04 (1.27) [0.92-4.72]	1.46 (0.11) [1.30-1.64]
	Brown Trout		
96 Hour Mortality (%)	7	---	0
Sub-Chronic Mortality (%)	7% - 6 days	---	10% - 8 days
	Brook Trout		
96 Hour Mortality (%)	0	---	0
Sub-Chronic Mortality (%)	0% - 7 days	---	10% - 8 days
	Cutthroat Trout		
96 Hour Mortality (%)	---	20	---
Sub-Chronic Mortality (%)	---	35% - 5 days	---

Table 26. Average total (T) and dissolved (D) metal concentrations (µg/L) in ambient, **untreated, Animas River water** and **experimental control water**. Standard deviations in parentheses and range in brackets. (Note: As and Se not reported, generally below Trace ICP detection limits [15 µg/L and 2 µg/L, respectively].

	Spring 1999		**Fall 1999**		**Early Spring 2000**	
Metals (µg/L)	Animas R.	Control	Animas R.	Control	Animas R.	Control
Al (T)	824 (382) [536-1717]	439 (153) [293-888]	1827 (272) [1640-2480]	1627 (286) [1255-2342]	678 (406) [490-1757]	497 (82.1) [398-668]
Al (D)	22.3 (23.8) [0.0-83]	17.8 (537) [6.0-30.0]	29.6 (8.8) [16-41]	55.2 (84.1) [17-293]	26.6 (8.2) [17-40]	27.2 (9.7) [18-48]
Cd (T)	1.0 (0) [1.0-1.0]	0.38 (0.51) [0.0-1.0]	4.65 (0.11) [4.5-4.8]	1.38 (0.56) [0.62-2.24]	1.56 (0.14) [1.39-1.74]	0.36 (0.39) [0.0-0.93]
Cd (D)	1.0 (0)[1.0-1.0]	0.38 (0.51) [0.0-1.0]	4.04 (1.27) [0.92-4.72]	1.28 (0.59) [0.52-2.24]	1.46 (0.11) [1.30-1.64]	0.33 (0.34) [0.0-0.80]
Cu (T)	25.7 (9.7) [18-48]	14.5 (3.48) [10.0-23.0]	49.3 (7.3) [44.4-66.3]	37.9 (4.6) [30.9-47.0]	35.8 (17.0) [27.6-80.6]	22.2 (2.79) [17.9-26.0]
Cu (D)	7.2 (2.9) [5-13]	5.6 (0.87) 5.0-8.0	12.0 (3.6) [9.1-20.3]	10.8 (2.45) [7.7-15.8]	10.1 (1.4) [8.6-13.3]	7.0 (0.48) [6.6-8.1]
Fe (T)	2003 (1157) [1179-4749]	896 (392) [578-2078]	2950 (479) [2629-4080]	2492 (536) [1776-3803]	1583 (1754) [859-6257]	951 (238) [697-1425]
Fe (D)	40.6 (20.4) [20-88]	36.5 (12.1) [25-72]	318 (157) [80-483]	146 (128) [46-484]	34.4 (15.4) [14-60]	32.6 (19.6) [12-68]
Mn (T)	338 (82.3) [276-530]	190 (71.3) [47-288]	1174 (62.6) [1063-1232]	962 (175) [658-1204]	436 (56.4) [366-546]	255 (133) [48-400]
Mn (D)	220 (15.5) [207-255]	150 (74.3) [21-248]	1168 (107) [959-1261]	987 (185) [663-1229]	408 (55.8) [329-474]	254 (141) [19-402]
Pb (T)	25.9 (15.1) [14-62]	11.2 (5.3) [7-26]	4.35 (1.51) [3.0-7.8]	3.93 (1.51) [2.60-7.50]	11.5 (15.7) [4.0-53.1]	6.34 (3.61) [3.3-12.2]
Pb (D)	0.11 (0.33) [0.0-1.0]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Zn (T)	245 (37.2) [207-328]	130 (113) [30-427]	643 (10.3) [624-656]	211 (79.2) [120-347]	370 (43.4) [334-476]	108 (73.4) [38-215]
Zn (D)	171 (11.7) [160-195]	102 (110) [15-383]	545 (174) [120-633]	188 (81.6) [98-322]	321 (24.7) [286-362]	93.7 (74.3) [11-193]

Table 27. EPA's hardness based, acute and chronic criteria (µg/liter) for copper, zinc, and cadmium at water hardness characteristics of the Animas River and laboratory control waters.

	EPA'S HARDNESS BASED CRITERIA (µg/L)					
HARDNESS mg/L	**COPPER**		**ZINC**		**CADMIUM**	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
40.8	7.62	5.50	54.8	49.6	1.43	0.56
42.3	7.88	5.67	56.4	51.1	1.49	0.58
45.0	8.36	5.98	59.5	53.9	1.59	0.61
51.3	9.46	6.68	66.5	60.2	1.85	0.67
52.6	9.68	6.83	67.9	61.5	1.90	0.68
54.6	10.0	7.05	70.1	63.5	1.98	0.70
81.3	14.6	9.91	98.2	88.9	3.10	0.96
210	35.7	22.3	219	199	9.06	2.03

Table 28. Average total metal concentrations (µg/L) in **laboratory control water**. Concentrations determined by trace ICP.

Metals (µg/L)	**Mean**	Std. Deviation	Range
Aluminum (Al)	44.7	18.8	33.2-66.4
Arsenic (As)	2.45	0.89	1.51-3.27
Cadmium (Cd)	0.0	0.0	0.0-0.0
Copper (Cu)	2.14	0.82	1.62-3.08
Iron (Fe)	11.5	5.3	5.79-16.2
Manganese (Mn)	0.9	0.5	0.5-1.4
Lead (Pb)	1.2	2.1	0.0-3.6
Selenium (Se)	0.0	0.0	0.0
Zinc (Zn)	2.23	1.82	0.65-4.22

Table 29. Average, standard deviations (in parentheses) and ranges of major water quality characteristics of **Animas River** water during toxicity tests conducted in the Spring 1999 before and after treatment with Chelex.

	Brown Trout (06/21/99-06/28/99)		Brook Trout (06/28/99-07/05/99)	
	Before Treatment	After Treatment	Before Treatment	After Treatment
Hardness (mg $CaCO_3$/L)	42.5 (0.9) 41.7-44.0	42.3 (0.5) 41.7-42.9	45.1 (2.1) 42.0-48.7	45.0 (2.2) 41.7-48.6
Alkalinity (mg $CaCO_3$/L)	15.1 (0.1) 15.0-15.2	15.6 (1.2) 13.6-19.2	15.3 (1.0) 14.4-17.6	14.7 (0.6) 13.6-16.8
pH (S.U.)	7.12 (0.15) 7.01-7.30	7.29 (0.09) 7.12-7.45	7.24 (0.11) 7.08-7.43	7.17 (0.16) 6.90-7.62
Dissolved Oxygen (mg /L)	6.69 (0.80) 6.07-7.59	7.27 (0.37) 6.46-8.01	8.31 (0.37) 7.68-8.69	7.39 (0.81) 5.8-9.2
Temperature (°C)	12.7 (2.4) 10.4-15.1	11.7 (1.5) 10.4-14.2	9.6 (2.1) 6.8-12.2	11.3 (2.6) 5.3-16.0
Conductivity (µS/cm)	88.8 (2.5) 86.0-90.3	87.4 (3.2) 82.4-93.1	86.8 (3.6) 82.1-90.2	89.3 (4.0) 77.8-101.6

Table 30. Average, standard deviations (in parentheses) and ranges of major water quality characteristics of **Animas River** water during toxicity tests conducted in the Fall 1999 before and after treatment with Chelex.

	Cutthroat Trout (10/15/99-10/23/99)	
	Before Treatment	After Treatment
Hardness ($mg\ CaCO_3/L$)	212 (8.8) 195-220	210 (11.0) 194-222
Alkalinity ($mg\ CaCO_3/L$)	15.1 (1.7) 13.6-17.4	13.8 (0.9) 12.4-16.4
pH (S.U.)	6.92 (0.10) 6.79-7.04	6.86 (0.07) 6.70-7.05
Dissolved Oxygen (mg /L)	10.54 (0.28) 10.3-11.0	9.27 (0.3) 8.6-10.0
Temperature (°C)	3.2 (1.9) 0.1-5.0	6.9 (0.7) 5.6-9.3
Conductivity (µS/cm)	---	—

Table 31. Average, standard deviations (in parentheses) and ranges of major water quality characteristics of **Animas River** water during toxicity tests conducted in Early Spring 2000 before and after treatment with Chelex.

	Brown and Brook Trout (05/06/2000-05/15/2000)	
	Before Treatment	After Treatment
Hardness (mg $CaCO_3$/L)	83.6 (3.9) 76.8-86.4	81.3 (7.5) 68.0-94.0
Alkalinity (mg $CaCO_3$/L)	14.3 (1.3) 12.0-16.0	13.8 (1.0) 12.0-16.0
pH (S.U.)	7.39 (0.28) 7.07-7.78	7.44 (0.22) 7.06-7.78
Dissolved Oxygen (mg /L)	8.17 (0.42) 7.6-8.7	7.90 (0.54) 6.8-8.7
Temperature (°C)	7.4 (1.4) 5.4-8.5	8.3 (1.5) 5.3-10.5
Conductivity (µS/cm)	158 (13) 140-180	145 (16) 110-170

Table 32. Average, standard deviations (in parentheses) and ranges of major water quality characteristics of **laboratory water** during toxicity tests with copper (Cu) and zinc (Zn) conducted in the spring and fall of 1999 water quality conditions similar to insitu toxicity test performed in the Animas River.

Water Quality	Species	Copper and Zinc - Spring 1999	
Hardness (mg $CaCO_3$/L)	Brown & Brook Trout	52.6 (0.2) 48.2 - 56.2	
Alkalinity (mg $CaCO_3$/L)		35.0 (0.9) 32.6 - 36.8	
pH (S.U.)		7.76 (0.07) 7.60 - 7.86	
Dissolved Oxygen (mg /L)		9.56 (0.23) 9.2 - 10.1	
Temperature (°C)		12.0 (0.2) 11.6 - 12.5	
Conductivity (µS/cm)		87.0 (2.0) 83.5 - 96.0	
		Copper - Fall 1999	**Zinc - Fall 1999**
Hardness (mg $CaCO_3$/L)	Cutthroat Trout	50.4 (2.3) 47.2 - 54.2	51.3 (2.4) 48.6 - 55.8
Alkalinity (mg $CaCO_3$/L)		38.0 (0.9) 36.8 - 39.6	38.2 (0.9) 36.6 - 39.0
pH (S.U.)		7.47 (0.25) 7.21 - 7.82	7.30 (0.11) 7.12 - 7.47
Dissolved Oxygen (mg /L)		10.67 (0.25) 10.1 - 11.0	10.65 (0.28) 9.8 - 11.0
Temperature (°C)		6.5 (0.2) 6.3 - 6.9	6.5 (0.2) 6.2 - 6.7
Conductivity (µS/cm)		78.6 (1.0) 77.4 - 81.2	79.5 (2.8) 76.1 - 85.7

Table 33. Average, standard deviations (in parentheses) and ranges of major water quality characteristics of **laboratory water** during toxicity tests with copper (Cu) and zinc (Zn) conducted in Early Spring 2000 water quality conditions similar to insitu toxicity test performed in the Animas River.

Water Quality	**Species**	**Copper - Early Spring 2000**	**Zinc - Early Spring 2000**
Hardness (mg $CaCO_3$/L)	Brown & Brook Trout	53.3 (1.7) 50.0 - 55.0	54.6 (2.6) 50.8 - 60.4
Alkalinity (mg $CaCO_3$/L)		37.3 (1.8) 34.6 - 40.0	37.6 (1.3) 35.8 - 39.4
pH (S.U.)		7.46 (0.13) 7.28 - 7.65	7.28 (0.19) 6.99 - 7.68
Dissolved Oxygen (mg /L)		8.71 (0.51) 7.6 - 9.3	9.08 (0.35) 8.2 - 9.5
Temperature (°C)		10.8 (0.4) 10.3 - 11.7	10.7 (0.3) 10.1 - 11.2
Conductivity (µS/cm)		86.7 (2.5) 82.1 - 89.8	88.1 (0.3) 83.1 - 98.4
		Copper - Early Spring 2000	**Zinc - Early Spring 2000**
Hardness (mg $CaCO_3$/L)	Cutthroat Trout	41.2 (1.7) 38.4 - 45.3	40.8 (1.8) 37.2 - 44.4
Alkalinity (mg $CaCO_3$/L)		30.9 (0.8) 30.0 - 32.4	31.6 (1.2) 29.0 - 34.6
pH (S.U.)		7.45 (0.18) 7.13 - 7.83	7.49 (0.17) 7.20 - 7.76
Dissolved Oxygen (mg /L)		9.06 (0.19) 8.7 - 9.6	9.23 (0.24) 8.7 - 9.8
Temperature (°C)		12.0 (0.3) 11.1 - 12.5	12.0 (0.2) 11.6 - 12.3
Conductivity (µS/cm)		75.0 (1.6) 71.4 - 77.5	75.2 (3.3) 61.8 - 81.7

Table 34. Mean lengths (mm) and weights (g) and standard deviation (in parentheses) of fish used in the seasonal Animas River onsite toxicity tests.

Season	Species	Length (mm)	Weight (g)
Animas River Toxicity Tests on Brown, Brook, and Cutthroat Trout			
Spring 1999	Brown Trout	66.7 (8.0)	2.838 (1.05)
Spring 1999	Brook Trout	74.4 (6.9)	3.557 (2.286
Fall 1999	Cutthroat Trout	34.0 (2.9)	0.241 (0.071)
Early Spring 2000	Brown Trout	46.3 (5.8)	0.849 (0.347)
Early Spring 2000	Brook Trout	29.1 (2.9)	0.153 (0.056)
Laboratory Toxicity Tests on Brown, Brook, and Cutthroat Trout			
Spring 1999	Brown Trout	92.1 (14.6)	8.92 (4.87)
Spring 1999	Brook Trout	119.4 (14.6)	16.16 (6.38)
Fall 1999	Cutthroat Trout	93.0 (10.2)	7.02 (2.38)
Early Spring 2000	Brown Trout	62.6 (8.0)	2.32 (1.02)
Early Spring 2000	Brook Trout	41.8 (4.7)	0.51 (0.18)
Early Spring 2000	Cutthroat Trout	74.1 (8.7)	3.62 (1.33)

STUDY PLAN B: LABORATORY STUDIES

Objective: To research and develop information on, or analytical tools in, aquatic chemistry and aquatic toxicology to better assess toxic responses of pollutants to aquatic life in laboratory and natural waters, such as the Arkansas River.

Job B1. Chemical Equilibria and Kinetic Effects on the Bioavailability and Toxicity of Metals to Aquatic Life

Job Objective: To develop analytical methods using Ion Chromatography, ion separation and/or ultrafiltration to measure toxic fractions and effects of chemical kinetics on toxicity of zinc, copper, lead, cadmium and/or silver to *Ceriodaphnia dubia*, rainbow trout, brown trout and/or fathead minnows in waters of different complexing capacity. Concurrently, investigate effects of chemical kinetics on results obtained from toxicity tests.

Job B2. Use of Biochemical Methods to Measure Disruption of Ion Regulation and Stress in Aquatic Organisms Exposed to Metals

Job Objective: To develop biochemical methods to measure effects on enzyme systems using electrophoresis or other methods to assess stress in rainbow and brown trout exposed to zinc, copper, lead and/or cadmium.

Job B3. Investigations on the Toxicity of Silver to Aquatic Organisms in Waters of Different Complexing Capacity

Job Objective: To develop acceptable toxicant concentrations of silver for cold- and warmwater fishes in hard high alkaline, and soft low alkaline waters and assess the toxicity of different silver compounds.

Job B4. Effects of Calcium Hardness, Inorganic and Organic Ligands and Sediments on Toxicity of Metals to Aquatic Organisms

Job Objective: To determine antagonistic effects of calcium hardness in low alkaline waters and the effects of specific inorganic and organic ligands and sediments on acute and long-term toxicity of zinc, copper, lead, cadmium, and/or silver to rainbow trout, brown trout and/or fathead minnows.

ACCOMPLISHMENTS:

See Job A2 above comparing results of laboratory toxicity tests to results from site specific tests on the toxcity of copper and zinc to brown, brook, and cutthroat trout in the Animas River below Silverton.

Job B5. Investigations on Enhanced Toxicity of Unionized Ammonia to Fish at Cold Water Temperatures

Job Objective: To determine effects of temperature on toxicity of unionized ammonia to rainbow trout and fathead minnows or other warmwater species at optimal and less than 5°C water temperatures.

Job B6. Effects of Episodic Exposure on Toxicity and Sensitivity of Aquatic Life to Intermittent Exposure to Metals

Job Objective: To determine toxic effects and organism sensitivity to intermittent exposure of zinc, copper, lead, and/or cadmium to rainbow trout, brown trout and/or fathead minnows, and their ability to acquire and/or lose tolerance.

Job B7. Investigations on Enhanced Toxicity of Water-Borne Metals to Aquatic Life Exposed to Dietary Sources of Metals

Job Objective: To determine effects of water-borne zinc, copper, cadmium, lead and/or manganese on their toxicity to rainbow and brown trout following and/or concurrent with exposure to dietary metals.

Job B8. Investigations on Effects and Interactions of Multiple Metal Exposure on Toxicity to Aquatic Life

Job Objective: To determine effects of exposure of rainbow trout and/or brown trout to zinc, copper, cadmium, lead, and manganese at different combinations found in Colorado's mining areas. Will require an ability to measure bioavailable forms on metals as outlined in Job B1.

ACCOMPLISHMENTS:

Toxicity test have been performed to evaluate possible synergistic toxcity relationships between copper and zinc using both brown and brook trout. Data from these experiments experiments are being analyzed and evaluated and will be reported next segment.

STUDY PLAN C: TECHNICAL ASSISTANCE

Objective:

To provide expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Division of Wildlife personnel, and other state and federal agencies.

ACCOMPLISHMENTS:

Job C1. Water Quality Assistance to Other Personnel

Job Objectives:

1. To oversee the training and evaluation of metal analysis by laboratory technicians.
2. To assist Division and other state and federal personnel in the analysis and toxicological assessment of water quality data.
3. To develop and maintain a quality assurance program to evaluate the quality of analytical results for metals.
4. To collect and analyze metals concentrations in samples from the Arkansas River.

Water quality characteristics and or metal analyses were performed for the following persons and agencies:

Phil Schler, Colorado Division of Wildlife (CDOW)
Mike Japhet, CDOW
John Woodling, CDOW
Barb Horn, CDOW
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