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EFFECT OF COPPER AND ZINC ON JUVENILE SALMONIDS EXPOSED TO SIMULATED COOLING TOWER BLOWDOWN WATER

by

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ABSTRACT

Proposed cooling water discharges into the Chehalis River from twin nuclear power plants under construction in Grays Harbor County, Washington may contain as much as 0.254 mg/l copper and 0.122 mg/l zinc. The sources of these metals are primarily corrosion products and concentrated makeup water. Under certain water quality conditions copper and zinc at these concentrations are toxic to aquatic biota.

The potential impact of the proposed discharge was investigated using standard 96-hour bioassays with 3 species of salmonids (rainbow trout, Salmo gairdneri; coho salmon, Oncorhynchus kisutch; and chinook salmon, Oncorhynchus tshawytscha). Bioassays were conducted with ambient Chehalis River water (simulated cooling tower blowdown). To compliment mortality data, atomic absorption spectrophotometry (AAS) and differential pulse anodic stripping (DPAS) were used to determine total and labile concentrations, respectively. In addition, hardness, alkalinity, and pH were determined for all test waters.

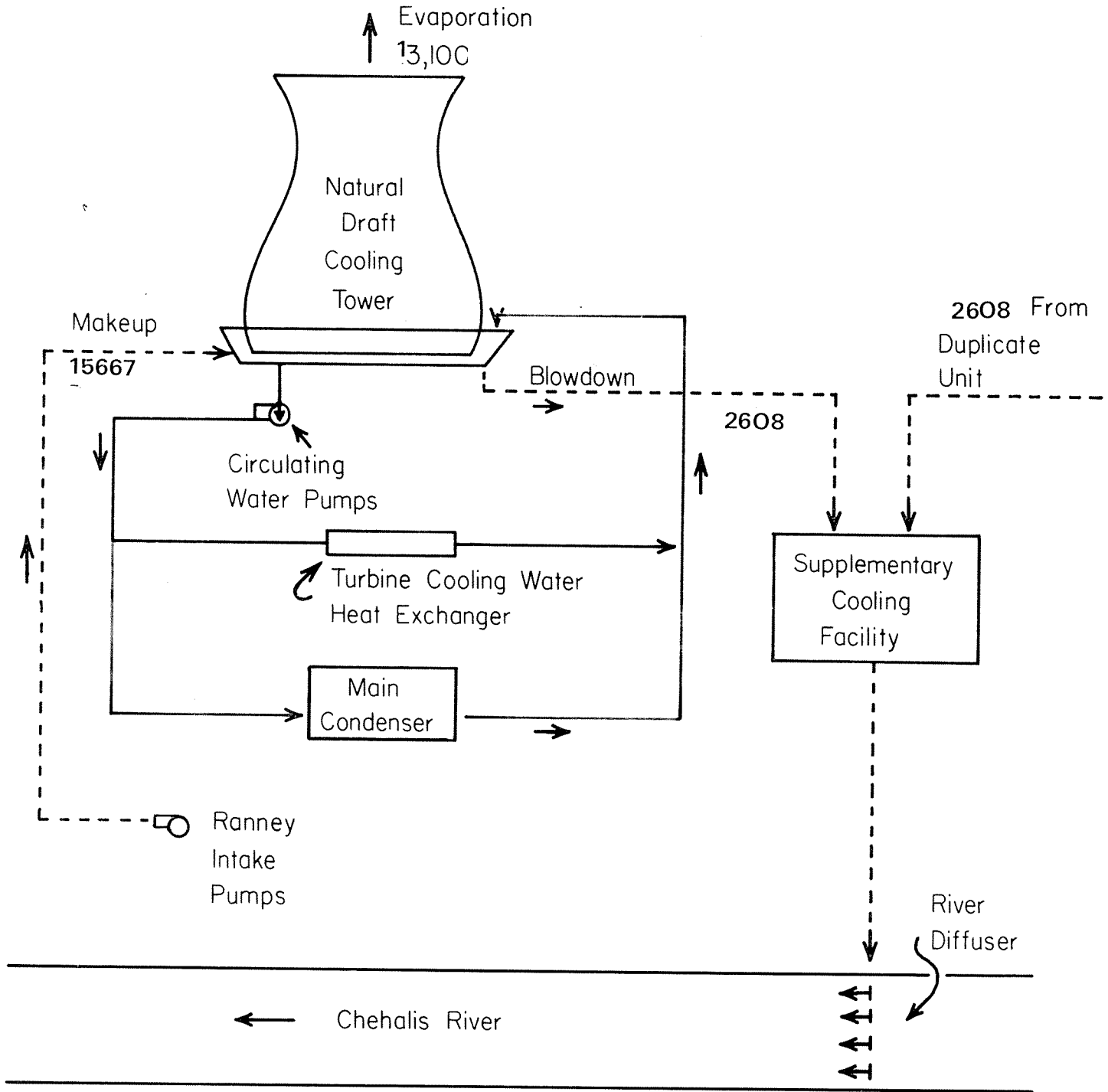
Ninety-six hour LC_{50} values in ambient river water for copper ranged from 0.139 to 0.242 mg/l. Tests with chinook salmon were the most sensitive (96-hour LC_{50} of 0.139 mg/l) followed by tests with rainbow trout (0.225 mg/l) and coho salmon (0.242 mg/l). For zinc, LC_{50} values were established for chinook salmon (0.094 mg/l) and rainbow trout (0.311 mg/l) only. LC_{50} values for copper in concentrated water were significantly higher than those in ambient river water ranging from 0.368 mg/l to 0.611 mg/l. Rainbow trout was the only species tested in concentrated water. Water quality data suggested that increased hardness and alkalinity resulted

in decreased toxicity; however, a clear relationship was not established. Analysis for labile copper in the tests with concentrated water indicated that concentrated water has a large capacity to complex ionic copper. Results showed that additions of 0.200 mg/l ionic copper resulted in labile copper concentrations of 0.050 to 0.060 mg/l as determined by DPAS.

The LC_{50} values and results of chemical analysis demonstrated the importance of chemical form and the effects of other water quality parameters on these forms in copper and zinc toxicity. Based on the collected data and projections of discharge water quality it was concluded that the discharge will not contain concentrations of the toxic forms of copper and zinc great enough to cause toxicity and that no impact will result from the proposed discharge.

INTRODUCTION

The Washington Public Power Supply System (WPPSS) is constructing twin nuclear power plants with a proposed power level of 1,242 megawatts electrical each. The construction site is located in southeastern Grays Harbor County, approximately 1.5 miles from the confluence of the Satsop and Chehalis rivers. At the rated power, each unit will produce 8.7×10^9 Btu/hr waste heat which will be transferred to cooling water circulating through the condensers and the cooling tower. These towers are concrete, hyperbolic natural-draft structures, approximately 500 ft tall and 510 ft in diameter at the base. The source of water, or makeup water, for the cooling water system will be the Chehalis River aquifer. The makeup water will be withdrawn from the aquifer by a subsurface collector system (Ranney collectors). Although most of the water is lost to the atmosphere due to evaporation during the cooling process, some water must also be continuously discharged, as blowdown water, to prevent the accumulation of dissolved solids in the system. Because of the large evaporative loss of water to the atmosphere, constituents of the river water will be concentrated approximately six times (6x) before discharge (Fig. 1). Two constituents of major concern are copper and zinc, which are considered toxic at or below 1 ppm under certain water quality conditions (Committee on Water Quality 1973; Sprague and Ramsey 1965). In addition to ambient levels being increased by concentration, corrosion products are expected to contribute to the total copper discharged from the system. As a result, it is estimated that the effluent may contain as much as 0.254 mg/l copper and 0.122 mg/l zinc. The effluent will be discharged into the Chehalis River through a multiport diffuser designed to allow for rapid mixing with receiving waters.



a) Flow rates are in gallons per minute

b) Dashed lines indicate makeup and blowdown

Figure 1. Schematic of single cooling tower unit (from Nuclear Regulatory Committee 1975).

It has been shown that the chemical species or form of copper present rather than the total concentration determines its toxicity (Committee on Water Quality 1973). Also, the toxicity of a given concentration of copper is in part dependent on other water quality conditions. Because no experimental data exist on copper toxicity in the Chehalis River in relation to these factors, a study has been conducted to determine the proposed discharge is potentially harmful to the aquatic biota. Using analytical and bioassay techniques the following four areas were investigated:

(1) Copper and zinc concentrations in ambient Chehalis River water.

(2) The copper complexing capacity in ambient river water and concentrated river water.

(3) The water quality of concentrated river water.

(4) The toxicity of copper and zinc in ambient and concentrated river water to three species of juvenile salmonids.

Ambient Chehalis River water studies (1) and complexing capacity studies (2) were conducted by Samuel Felton, Fisheries Research Institute, and are discussed in a separate report (Felton 1978). This report deals primarily with the water quality of concentrated water (3) and its relation to copper and zinc toxicity.

EXPERIMENTAL DESIGN, METHODS AND MATERIALS

A total of 26 bioassays using over 2,500 fish was conducted from 26 June 1977 to 27 June 1978 at the Fisheries Research Institute, University of Washington, Seattle, Washington. Except when noted, standard

analytical and bioassay techniques (American Public Health Association et al., 1977) were employed in all experiments.

In describing the various bioassays and water samples, the following terms with their explanations are used:

Concentrated water - a sample of Chehalis River or well water which has been reduced in volume by evaporation to simulate the concentration in the cooling tower. Volume reductions were generally to 1/6 of the original volume (6x); however, 1/12 (12x), or 1/18 (18x) were also used.

Ambient river water or river water - unaltered Chehalis River water.

Ionic addition - the addition of copper or zinc sulfate to elevate copper or zinc levels in test water.

Total copper - all copper in a sample, regardless of chemical form, as determined by atomic absorption spectrophotometry (AAS).

Labile copper - all copper in the free or unbound form (Cu^{++}), plus any loosely bound forms which may be detected by differential pulse anodic stripping (DPAS).

Bioassays

Bioassays are referred to as concentrated water bioassays or ionic addition bioassays depending on the manner in which test solutions were prepared. A summary of the bioassays conducted is presented in Table 1.

Concentrated Water Bioassays

The purpose of the concentrated water bioassays was to determine if water concentrated 6x, 12x, or 18x was acutely toxic to fish. Water was concentrated by evaporation in a large stainless steel container (Fig. 2). To simulate the cooling tower as closely as possible, water temperature during concentration was maintained at approximately 45°C (113°F) by two

Table 1. Summary of bioassay experiments.

Bioassay no.	Starting date	Species tested	Total no. of fish used	Mean water temp. (°C)	Design-Purpose
1	6-6-77	Rainbow trout	36	16.0	Concentrated water bioassay (6x)
2	6-6-77	Coho salmon	36	16.0	Concentrated water bioassay (6x)
3	6-13-77	Rainbow trout	36	18.9	Concentrated water bioassay (6x)
4	6-13-77	Coho salmon	36	18.9	Concentrated water bioassay (6x)
5	6-27-77	Coho salmon	75	19.5	Ionic addition bioassay - determine 96-hr LC50 for Cu in ambient water
6	7-11-77	Rainbow trout	60	19.0	Concentrated water bioassay (6x, 12x)
7	7-11-77	Rainbow trout	60	19.1	Ionic addition bioassay - determine 96-hr LC50 for Zn in ambient water
8	7-18-77	Rainbow trout	60	19.5	Concentrated water bioassay (6x, 12x)
9	7-19-77	Rainbow trout	75	20.0	Ionic addition bioassay - determine 96-hr LC50 for Zn in ambient water
10	8-1-77	Coho salmon	75	21.5	Ionic addition bioassay - determine 96-hr LC50 for Cu in ambient water
11	8-9-77	Coho salmon	60	24.0	Ionic addition bioassay - determine 96-hr LC50 for Zn in ambient water
12	10-31-77	Rainbow trout	120	12.5	Ionic addition bioassay - determine 96-hr LC50 for Cu in ambient water
13	10-31-77	Rainbow trout	120	12.5	Ionic addition bioassay - determine 96-hr LC50 for Zn in ambient water
14	11-1-77	Rainbow trout	80	12.2	Concentrated water bioassay (6x, 12x)
15	11-15-77	Rainbow trout	80	10.0	Concentrated water bioassay (6x, 12x)
16	12-2-77	Rainbow trout	140	9.9	Concentrated water bioassay (6x, 12x, 18x)
17	12-5-77	Rainbow trout	120	9.5	Ionic addition bioassay - determine 96-hr LC50 for Cu in ambient water

Table 1. continued.

Bioassay no.	Starting date	Species tested	Total no. of fish used	Mean water temp. (°C)	Design-Purpose
18	12-5-78	Rainbow trout	140	9.5	Ionic addition bioassay - determine 96-hr LC50 for Zn in ambient water
19	1-16-78	Chinook salmon	120	7.9	Ionic addition bioassay - determine 96-hr LC50 for Cu in ambient water
20	1-16-78	Chinook salmon	120	7.9	Ionic addition bioassay - determine 96-hr LC50 for Zn in ambient water
21	1-23-78	Chinook salmon	120	7.5	Ionic addition bioassay - determine combined toxicity of Cu and Zn ambient water'
22	2-14-78	Chinook salmon	260	7.8	Ionic addition bioassay - determine combined toxicity of Cu and Zn in ambient water
23	5-26-78	Rainbow trout	180	14.6	Ionic addition bioassay - determine 96-hr LC50 for Cu in concentrated water
24	6-5-78	Rainbow trout	200	19.8	Ionic addition bioassay - determine 96-hr LC50 for Cu in concentrated water
25	6-15-78	Rainbow trout	160	17.4	Ionic addition bioassay - determine 96-hr LC50 for Cu in concentrated water
26	6-27-78	Rainbow trout	180	17.4	Ionic addition bioassay - determine 96-hr LC50 for Cu in concentrated water

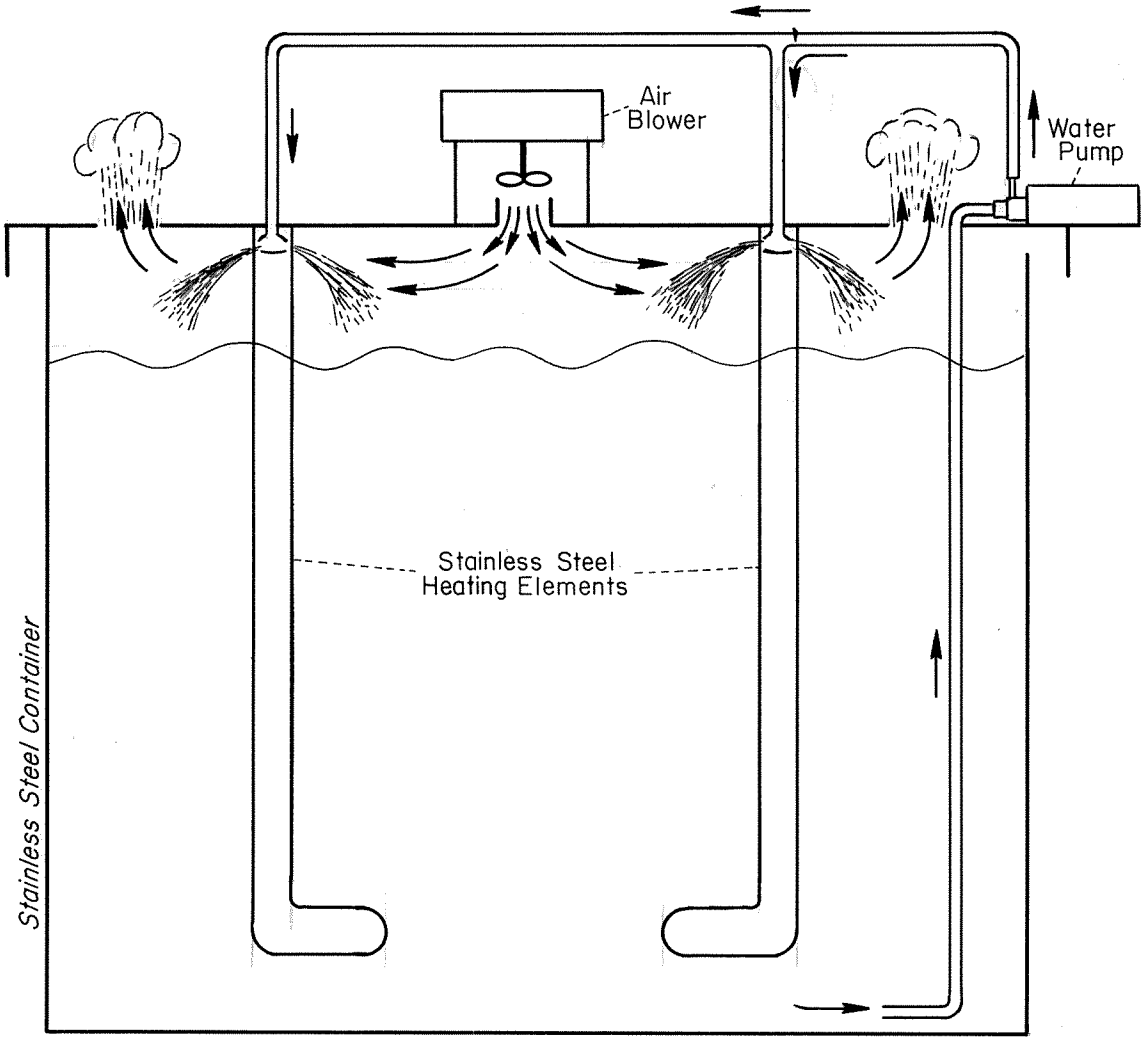


Fig. 2. Stainless steel concentrator used in simulating blowdown water.

stainless steel heating elements suspended in the water. Water was circulated by a non-metallic pump which drew water from the bottom of the tank and sprayed it across the surface. Ventilation, or draft, was maintained by an air blower which blew steam away from the container. Water was then evaporated until the desired volume reduction had been reached.

A total of 9 concentrated water bioassays was conducted (Nos. 1-4, 6, 8, 14-16). In bioassay Nos. 1-4, water was concentrated 6x for bioassay. In bioassay Nos. 6, 8, 14, and 15, the water was concentrated 12x, and then bioassayed. To simulate seasonal peaks observed in ambient copper and zinc levels in Chehalis River, water for bioassay No. 16 was spiked with copper sulfate and zinc sulfate to 40 ppb (the highest ambient levels recorded during the study), and then concentrated 6x, 12x, and 18x. A grab sample for chemical analysis was taken after concentration before the bioassay was started. Analysis for copper and zinc in these samples showed that ionic additions made in this manner, i.e., before concentration, resulted in a significant "loss" or precipitation of copper and zinc, and expected levels of copper in solution were never observed after concentration. Large deposits of sediment were observed to collect in the bottom of the concentrating tank during concentration and these sediments may act as a matrix for residual metals, including copper and zinc.

Detailed information regarding test conditions can be found in the Appendix (Tables A1-A19).

Ionic Addition Bioassays

The purpose of the ionic addition bioassays was to determine 96-hour LC_{50} values for fish exposed to ambient river water or concentrated water

spiked with ionic copper and/or zinc. Spikes were made with stock solutions of 393 mg/l Cu SO_4-$5H_2O$ (100 mg/l Cu) and 439 mg/l Zn SO_4-$7H_2O$ (100 mg/l Zn).

In the first series of tests (Nos. 5, 7, 9-13, 17-22), ionic copper and zinc were added directly to Chehalis River water. Because of reported synergism of copper and zinc toxicity at higher concentrations (Sprague 1964; Sprague and Ramsey 1965; Lloyd 1961), LC_{50} values were first determined for test fish exposed to just copper or zinc alone and finally for fish exposed to both copper and zinc at levels proportional to background river levels.

In the second series of addition tests (Nos. 23, 24, 25, 26), ionic copper was added to concentrated water (addition made after concentration). In three of these bioassays, small amounts of ionic copper and zinc were also added previous to concentration to elevate ambient river concentrations. This did not always contribute to the total copper or zinc insult in the bioassay. Chemical analysis demonstrated that most of the copper added previous to concentration was not recovered after concentration.

The pH of Chehalis River water increased from 7.0 to 8.2 when water was concentrated to 6x. Preliminary tests and the literature (Andrew 1976; Shaw and Brown 1974; Committee on Water Quality 1973; Chapman 1977) indicate that pH will affect copper toxicity by altering the equilibrium of ionic copper in test water; therefore, in order to maintain control of the pH in the bioassay, the pH of the test solutions from the series using concentrated water was lowered from 8.2 to 7.0 with sulfuric acid before testing.

All exposure concentrations used for LC_{50} determinations calculated from chemical analysis of background copper and zinc levels and known quantities added. LC_{50} values were obtained either through the use of a computer program or graphically. The computer program, BMD03S (Dixon 1970), was used in all bioassays which resulted in at least three treatments with a partial kill. In other tests percent mortality (on a probit scale) was plotted against concentration (on a log scale) and a line was drawn by visual inspection. Both methods are in agreement with Finney's (1974) discussion of quantal bioassay data. Examples of the log-probit graphs are shown in Figs. 3 and 4. Due to small amounts of data, confidence limits on these values were not computed. Detailed information regarding conditions and exposure concentrations for each bioassay can be found in the Appendix (Tables A10-A26).

Water Quality and Copper Determinations

Because the toxicity of copper and zinc to fish may be dependent on the various water quality parameters, pH, hardness, alkalinity, total copper, and total zinc were routinely determined for all samples of river water used in the bioassays. In those bioassays where concentrated water was used, the various parameters were determined before and after concentration. Routine analysis of each test solution in each bioassay was not done because of the large number of bioassays conducted; however, two bioassays were conducted in which a grab sample was taken from each test container and analyzed for labile copper and zinc (Nos. 24 and 25).

Total copper and zinc measurements were done on a Perkin-Elmer 290 atomic absorption spectrophotometer. Relative error (the difference

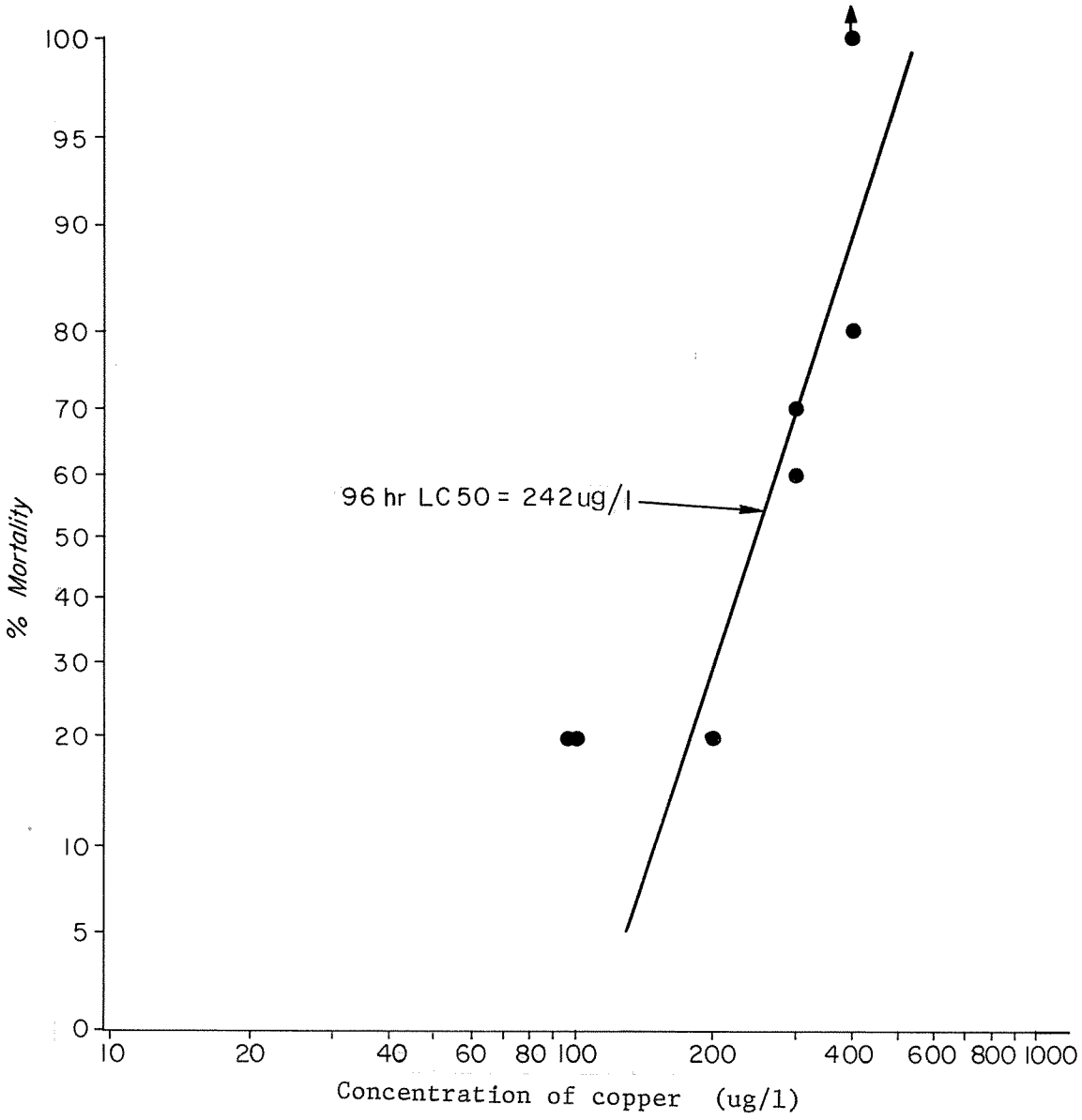


Figure 3. A graph of total mortality vs. concentration on a log-probit scale for rainbow trout exposed to ionic copper (bioassay no. 15).

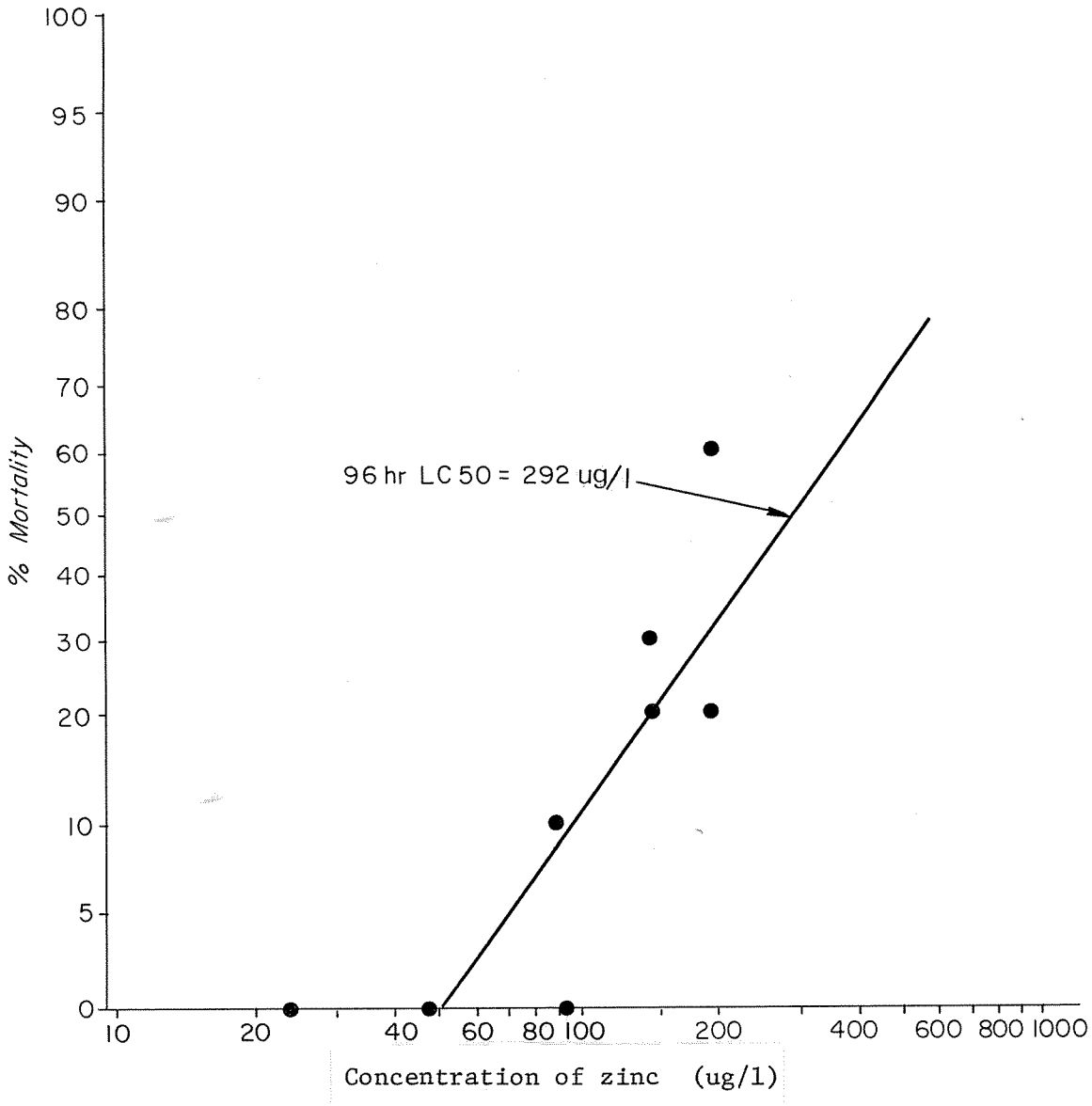


Figure 4. A graph of total mortality vs. concentration for rainbow trout exposed to ionic zinc (bioassay no. 18).

between mean of test results and the known standard expressed as a percentage of the known standard) and relative standard deviation (standard deviation expressed as a percentage of the mean) were determined according to Standard Methods for the Examination of Water and Wastewater (American Public Health Association et al., 1971) and calculated to be 3.4% and 11.2% at 1 ppm, respectively. Instrument sensitivity is to 0.025 mg/1. Because most samples contained copper and zinc concentrations below the sensitivity of the instrument, samples were concentrated by evaporation at low heat in a Brinkman Rotavap before analysis. The relative standard error at 3 ppb was calculated to be 53.9%. Percent recovery after concentration on the Rotavap was estimated to be 95%.

Ionic copper and zinc measurements were done on a Princeton Applied Research Model 374 Polarograph operating in the differential pulse anodic stripping (DPAS) mode. Sensitivity of this instrument is to 0.001 mg/1; thus, no concentrating of samples was required. Relative error and relative standard deviation were calculated to be 7.8% and 5.1% at 3500 na, respectively. Methyl orange procedures listed in Methods of Chemical Analysis of Water and Wastes (EPA 1971) were employed for alkalinity determinations. Hardness was calculated according to formulas found in Standard Methods for the Examination of Water and Wastewater (American Public Health Association et al., 1977). The calcium and magnesium values required for these calculations were determined by Perkin-Elmer 290. Temperature of test solutions during the bioassay was determined daily by a glass thermometer with a precision of 0.1°C.

Source of Water for Testing

Makeup water to be used during actual plant operation was unavailable because the Ranney wells were not yet constructed at the time of the study. During operation, most of the water will most likely originate from the river and pass through the groundwater system to the Ranney collector. Partly for these reasons, water, taken directly from the Chehalis River, was used as dilution and control water in all but two bioassays, which used well water pumped from the vicinity of the proposed Ranney system intake.

Ambient Chehalis River water was used as a control in all bioassays. Because the test fish were held in Lake Washington water prior to testing, it was used as an additional control in the first four bioassays.

During the study, river water was collected weekly and transported to the University in 30-gallon polyethylene drums. Table 2 summarizes the water quality of the water used in the bioassays.

Test Fish

Zero-age rainbow trout (*Salmo gairdneri*), coho salmon (*Oncorhynchus kisutch*), and chinook salmon (*O. tshawytscha*) were used in the bioassays. They were chosen because they are sensitive to copper and zinc toxicity, are commercially and recreationally important, are found seasonally near the proposed point of discharge, and were readily available from local sources listed in Table 3. Selection of fish for individual tests was determined by availability at the time. Table 4 summarizes the length and weight characteristics of the fish used.

Table 2. Mean water quality parameters for Chehalis River water used in bioassays.

	Mean	S. D.	Range	n
pH	7.0	0.3	6.5 - 7.5	12
Hardness (mg/l)	20.3	3.4	14.6 - 25.3	14
Alkalinity (mg/l)	22.0	8.7	13.0 - 33.7	12
Calcium (mg/l)	4.7	0.7	3.1 - 5.6	14
Magnesium (mg/l)	2.0	0.41	1.6 - 2.4	14
Total copper (mg/l)	0.004	0.003	N.D. - 0.008	15
Total zinc (mg/l)	0.006	0.002	0.002 - 0.009	16

N.D.: not detectable.

Table 3. Test fish used in copper-zinc toxicity tests.

Species	Age	Source of fish	Bioassay
Rainbow trout *(Salmo gairdneri)*	0-age	Washington State Dept. of Game, Puyallup Trout Hatchery, Puyallup, WA	1,3,6,7,8,9
Rainbow trout	0-age	Washington State Dept. of Game, Tacoma Game Farm, Tacoma, WA	12,13,14,15,16,17,18
Rainbow trout	0-age	College of Fisheries, Univ. of Wash., Seattle, WA	23,24,25,26
Coho salmon *(Oncorhynchus kisutch)*	0-age	College of Fisheries Univ. of Wash., Seattle, WA	2,4,5,10,11
Chinook salmon *(Oncorhynchus tshawytscha)*	0-age	College of Fisheries Univ. of Wash., Seattle, WA	19,20,21,22

Table 4. Length-weight data of test fish used in copper-zinc bioassays.

Bioassay no.	Species	Average total length (cm)	Average weight (g)	No. of fish in subsample
1	Rainbow trout	5.5	2.8	10
2	Coho	4.8	1.1	25
3	Rainbow trout	6.5	2.3	11
4	Coho	5.2	.7	13
5	Coho	6.1	2.1	68
6	Rainbow trout	7.0	3.9	35
7	Rainbow trout	6.6	4.4	22
8	Rainbow trout	7.7	6.8	19
9	Rainbow trout	7.7	5.8	14
10	Coho	6.9	3.5	22
11	Coho	7.6	3.9	13
12,13,14	Rainbow trout	3.9	0.5	33
15	Rainbow trout	4.2	0.6	19
16,17,18	Rainbow trout	3.9	0.5	19
19,20	Chinook	3.3	0.4	20
21	Chinook	3.5	0.4	20
22	Chinook	4.1	0.5	20
23	Rainbow trout	3.7	0.4	19
24,25,26	Rainbow trout	4.1	0.7	10

Acclimation to laboratory conditions took place in holding troughs supplied with a continuous flow of Lake Washington water. Fish were not fed two days prior to testing.

Test Containers

All bioassays were conducted in one-gallon, wide-mouth glass jars. Preparation of test containers included an acetone and an acid rinse. Preliminary bioassays done without aeration showed that dissolved oxygen (DO) levels in the test containers fell as low as 2.9 ppm within 24 hours and remained at these low levels, thereby affecting results. During the actual bioassays all test tanks were aerated by small glass tubes connected to air pumps with tygon tubing. Temperature control during the bioassay was maintained by bathing test tanks in a continuous flow of Lake Washington water; therefore, fish were subject to the daily and seasonal temperature fluctuations of Lake Washington. Mean water temperatures, determined from daily readings, are listed in Table 1.

RESULTS

Water Quality

Water quality of the ambient water used in the bioassays has already been presented in Table 2. Mean total copper and zinc concentrations were 0.004 mg/l and 0.006 mg/l, respectively, and maximum concentrations were 0.008 mg/l copper and 0.009 mg/l zinc. U.S. Geological Survey data (1961-1973) indicate maximum and mean ambient river dissolved copper values of 0.460 mg/l and 0.015 mg/l, respectively. For dissolved zinc, the

the values are 0.340 mg/l and 0.020 mg/l, respectively. The maximum values are considerably higher than the more recent data. Data collected by Sam Felton (1978), based on 44 weekly samples, resulted in mean and maximum total copper values of 0.007 mg/l and 0.042 mg/l, respectively. The differences between these more recent data and the past U.S.G.S. data may be due, in part, to variations in analytical techniques. Mean hardness and alkalinity values were 20.3 mg/l (as $CaCO_3$) and 22.0 mg/l (as $CaCO_3$), respectively. Ambient pH values ranged from 6.5 to 7.5 with a mean of 7.0.

The effect of concentrating river water on alkalinity and hardness was to raise them approximately proportional to the degree of concentration; however, copper and zinc levels never reached expected values (Table 5). Mean copper and zinc values for concentrated water were 0.010 mg/l and 0.014 mg/l, respectively. These data are presented for individual samples in Tables 6-12. From Tables 8, 10, and 12 it can be seen that analysis for total copper and zinc did not reflect quantities added, even when analysis was done immediately after making a test solution.

Two bioassays (Nos. 24 and 25) were conducted in which polarographic techniques (DPAS) were used to determine labile copper levels in test solutions after additions of ionic copper (copper sulfate) were made to concentrated water. Tables 13 and 14 present these data. The tables show that concentrated water has the property to bind up much of the free copper in a system. Additions of 0.200 mg/l ionic copper in the two samples resulted in labile copper concentrations of 0.055 mg/l (No. 24) and 0.058 mg/l (No. 25).

Table 5. Mean water quality parameters for simulated blowdown water (Chehalis River water concentrated 6 times).

	Mean	S. D.	Range	n
pH	8.3	0.01	7.9 - 8.6	6
Hardness (mg/l)	111.0	18.3	76.6 - 131.6	7
Alkalinity (mg/l)	119.7	29.2	83,5 - 155.0	6
Calcium (mg/l)	26.8	16.2	18.8 - 34.9	7
Magnesium (mg/l)	10.0	1.7	7.6 - 12.3	7
Total copper (mg/l)	0.010	0.009	N.D. - 0.016	7
Total zinc (mg/l)	0.014	0.006	0.008 - 0.021	6

Table 6. The effects of concentrating Chehalis River water used in bioassay no. 14 on water quality.

Water quality parameters	Ambient river water	Concentrated 6 times	Concentrated 12 times
pH	7.1	8.4	8.8
Hardness (mg/l)	23.6	131.6	194.0
Alkalinity (mg/l)	21.7	144.0	182.0
Calcium (mg/l)	5.6	34.9	49.0
Magnesium (mg/l)	2.4	10.8	17.7
Total copper (mg/l)	0.003	0.015	0.019
Total zinc (mg/l)	0.008	0.008	0.032

Table 7. The effects of concentrating Chehalis River water used in bioassay no. 15 on water quality.

Water quality parameters	Ambient river water	Concentrated 6 times	Concentrated 12 times
pH	7.0	7.9	8.4
Hardness (mg/l)	22.5	107.0	162.0
Alkalinity (mg/l)	21.7	107.0	215.0
Calcium (mg/l)	5.6	24.3	38.6
Magnesium (mg/l)	2.4	11.1	15.0
Total copper (mg/l)	0.003	0.016	0.011
Total zinc (mg/l)	0.005	0.008	0.032

Table 8. The effects of concentrating Chehalis River water used in bioassay no. 16 on water quality.

Water quality parameter	Ambient river water	Spiked*	Concentrated 6 times	Concentrated 12 times	Concentrated 18 times
pH	6.9		8.0	8.4	8.9
Hardness (mg/l)	19.4		110.0	183.5	319.0
Alkalinity (mg/l)	33.7		83.5	151.0	200.0
Calcium (mg/l)	4.8		18.8	32.8	43.9
Magnesium (mg/l)	1.8		8.9	16.9	22.2
Total copper (mg/l)	0.003	0.027	0.073	0.133	0.162
Total zinc (mg/l)	0.005	0.040	0.054	0.083	0.104

*Ionic copper and zinc added to ambient river water at levels of 0.037 mg/l (expected total of 0.040 mg/l) Cu and 0.035 mg/l (expected total of 0.040 mg/l) Zn.

Table 9. The effects of concentrating Chehalis River water used in bioassay no. 23 on water quality.

Water quality parameter	Ambient river water	Spiked*	Concentrated 6 times (6x)
pH	7.2		-
Hardness (mg/l)	17.4		104.0
Alkalinity (mg/l)	13.0		136.0
Calcium (mg/l)	4.2		27.0
Magnesium (mg/l)	1.7		9.4
Total copper (mg/l)	0.006	0.059	0.158
Total zinc (mg/l)	0.008	0.062	0.084

*Ionic copper and zinc added to ambient river water at levels of 0.044 mg/l (expected total of 0.050 mg/l) Cu and 0.042 mg/l (expected total of 0.050 mg/l) Zn.

Table 10. The effects of concentrating Chehalis River water used in bioassay no. 24 on water quality.

Water quality parameter	Ambient river water	Spiked*	Concentrated 6 times (6x)
pH	7.0		8.3
Hardness (mg/l)	22.2		123.0
Alkalinity (mg/l)	32.0		155.0
Calcium (mg/l)			29.0
Magnesium (mg/l)	2.7		12.3
Total copper (mg/l)	0.005	0.013	0.079
Total zinc (mg/l)	0.004	0.045	0.101

*Ionic copper and zinc added to ambient river water at levels of 0.045 mg/l (expected total of 0.050 mg/l) Cu and 0.045 mg/l (expected total of 0.050 mg/l) Zn.

Table 11. Effects of concentrating Chehalis River water used in bioassay no. 25 on water quality.

Water quality parameters	Ambient river water	Concentrated 6 times
pH	7.2	8.4
Hardness (mg/l)	14.6	76.6
Alkalinity (mg/l)	-	
Calcium (mg/l)	3.1	20.2
Magnesium (mg/l)	1.6	7.6
Total copper (mg/l)	N.D.	N.D.
Total zinc (mg/l)	0.007	0.021

Table 12. Effects of concentrating Chehalis River water used in bioassay no. 26 on water quality.

Water quality parameters	Ambient river water	Spiked*	Concentrated 6 times
pH	7.5		8.6
Hardness (mg/l)	19.4		125.0
Alkalinity (mg/l)	-		93.0
Calcium (mg/l)	4.6		33.4
Magnesium (mg/l)	1.7		10.1
Total copper (mg/l)	N.D.	.045	.111
Total zinc (mg/l)	0.006		.012

*Ionic copper was added to ambient river water at a level of 0.050 mg/l.

Table 13. Measurable labile copper in simulated blowdown water with additions of copper sulfate (bioassay no. 24) at pH = 7.

	Measurable ionic copper (mg/l)	Calculated total copper (mg/l)
Ambient	N.D.	0.005
Spiked ambient	N.A.	0.013
Concentrated (6x)	N.A.	0.079
6x + 0.200 mg/l	0.055	0.279
6x + 0.300 mg/l	0.113	0.379
6x + 0.400 mg/l	0.138	0.479
6x + 0.500 mg/l	0.156	0.579
6x + 0.600 mg/l	0.282	0.679
6x + 0.700 mg/l	0.342	0.779
6x + 0.800 mg/l	0.382	0.879

N.D.: Not detectable.

N.A.: Not available.

Table 14. Measurable labile copper in simulated blowdown water with additions of copper sulfate (bioassay no. 25) at pH = 7.

	Measurable ionic copper (mg/l)	Calculated total copper (mg/l)
Ambient	N.D.	N.D.
Concentrated (6x)	N.D.	N.D.
6x + 0.050 mg/l	0.007	0.050
6x + 0.100 mg/l	0.013	0.100
6x + 0.200 mg/l	0.058	0.200
6x + 0.400 mg/l	0.087	0.400
6x + 0.600 mg/l	0.255	0.600
6x + 0.800 mg/l	0.239	0.800

N.D.: Not detectable.

Bioassay Mortality

In the concentrated water bioassays, fish tested (rainbow trout and coho salmon) did not show an acute response to water concentrated up to 18x. Toxic forms of copper and zinc were not elevated sufficiently during the simulation process to induce mortality. In bioassay No. 16 in which spikes were added previous to concentration, copper and zinc levels rose significantly higher than other tests (0.162 mg/1 copper and 0.104 mg/1 zinc); however, no mortalities were incurred in the bioasssy. With the exception of one mortality in one 6x test solution, there were no mortalities in any concentrated water bioassay.

Because no mortality occurred in the concentrated water bioassays, no reference points of copper toxicity could be established. In the ionic addition bioassays, reference points (LC_{50} values) were established for copper toxicity in ambient Chehalis River water and concentrated water. Tables 15 and 16 list 96-hour LC_{50} values for several bioassays. Many of the bioassays are not included here because either the lethal response was not great enough, or a wide enough range of copper concentrations was not bioassayed to establish an LC_{50} value.

Among the species tested chinook salmon showed the greatest sensitivity to both copper and zinc in ambient river water. This greater sensitivity may be the result of inherent species differences, or more likely differences in water quality characteristics. For copper bioassays, 96-hour LC_{50} values ranged from 0.139 mg/1 (chinook) to 0.242 mg/1 (coho). For zinc in ambient river water, 96-hour LC_{50} values ranged from 0.094 mg/1 (chinook) to 0.311 mg/1 (rainbow).

Table 15. 96-hr LC50 values for copper addition bioassays.

Bioassay no.	Species	Hardness (mg/l)	Alkalinity (mg/l)	pH	96-hr LC50 (mg/l)
22a (ambient river water)	Chinook	15.3	19.9	7.4	0.139
19 (ambient river water)	Chinook	19.6	27.0	6.7	0.210
17 (ambient river water)	Rainbow	22.6	27.0	N.A.*	0.225
5 (ambient river water)	Coho	19.4	33.7	6.9	0.242
23 (concentrated 6x)	Rainbow	104.0	136.0	7.0	0.611
25 (concentrated 6x)	Rainbow	76.6	131.0	7.0	0.380
26 (concentrated 6x)	Rainbow	92.3	126.3	7.0	0.368

*N.A. = not available.

Table 16. 96-hr LC50 values for zinc addition bioassays.

Bioassay no.	Species	Hardness (mg/l)	Alkalinity (mg/l)	pH	96-hr LC50 (mg/l)
20 (Ambient river water)	Chinooks	19.6	27.0	6.7	0.094
22b (Ambient river water)	Chinooks	15.3	19.9	7.4	0.155
18 (Ambient river water)	Rainbows	19.4	33.7	6.9	0.292
13 (Ambient river water)	Rainbows	23.6	21.7	7.1	0.311

Table 17. 96-hr LC50 values for tests with both copper and zinc were added to Chehalis River water at levels proportional to those in ambient water.

Bioassay no.	Species	Hardness (mg/l)	Alkalinity (mg/l)	pH	96-hr LC50 (mg/l) Cu	96-hr LC50 (mg/l) Zn
22c (Ambient river water)	Chinooks	15.3	19.9	7.4	0.085	0.047
21 (Ambient river water)	Chinooks	25.3	28.5	6.5	0.143	0.095

Two tests were conducted with chinook salmon exposed to both copper and zinc. LC_{50} values for these bioassays are presented in Table 17. Bioassay No. 22c was part of a bioassay in which three series of insults were tested, these being copper only (22a), zinc only (22b), and both copper and zinc (22c). If the results of bioassay No. 22c are compared with Nos. 22a and 22b, the results suggest that copper and zinc toxicity at these concentrations are not significantly synergistic. To show this quantitatively, the LC_{50} values from No. 22c are expressed as fractions of the LC_{50} values for the individual toxicants and summed:

$$\frac{85}{139} + \frac{47}{155} = 0.91$$

A sum of exactly 1 would indicate additive toxicity, whereas an LC_{50} value of less than 1 would indicate a synergistic interaction.

In the concentrated water, 96-hour LC_{50} values for rainbow trout were much higher than those in ambient water, ranging from 0.368 mg/l copper (No. 26) to 0.611 mg/l copper (No. 23) (Table 15). For bioassay No. 25 an LC_{50} of 0.115 mg/l ionic copper, and 0.380 mg/l total copper was obtained. An LC_{50} value was not computed for bioassay No. 24 because there was an insufficient range of concentrations tested for probit analysis.

DISCUSSION

Prediction of the chemical quality of the cooling tower blowdown water is difficult because of the many variables. Nevertheless, theoretical calculations based on manufacturers' corrosion rates and ambient concentration indicate that the plant effluent could contain as much as 0.254 mg/l copper and 0.122 mg/l zinc. This projection assumes that no copper will

precipitate and settle in the cooling tower basin. It is doubtful that this will be the case. The free copper ion can undergo several reactions under the conditions present in the cooling tower which will result in insoluble forms of copper. Copper is known to react with hydroxides and bicarbonates to form malachite, an insoluble precipitate:

$$2Cu^{++} + 2H_2O + HCO_3 \leftrightharpoons Cu_2(OH)_2CO_3 + 3H^+$$

(Stiff 1971). In addition, a substantial percentage of the corrosion product is expected to be particulate copper, which will remain insoluble under the high pH conditions expected in the cooling tower basin. It follows that much of the copper in the cooling tower will settle in the basin and never be discharged. Furthermore, corrosion is expected to decrease over time as the surfaces of the corrosion sources become oxidized. Theoretical projections (Dr. James Roetzer, Envirosphere chemist, personal communication) based on conditions existing in the cooling tower indicate that at most, 25% of the copper in the discharge will be in the ionic form.

The small amount of empirical data gathered to date supports the above conclusions. Stratton and Lee (1975) examined acid-soluble copper concentrations in cooling tower makeup and basin water from 11 different sites. The mean copper values in blowdown ranged from 0.013 to 1.080 mg/l. The makeup water was generally low in copper and the main source was copper construction materials in the heat exchange system. Data from the Centralia power station yielded a total copper concentration of 0.065 mg/l on both sides of the condenser tubing, indicating less significant contributions from corrosion (Mulvihill and Kruger 1976). Observations made at

the Trojan Nuclear Power Plant at startup and at one year show a decline in corrosion rates. Total copper measurements at the inlet and outlet of the condenser tubing were 2.61 and 2.69 mg/l, respectively, for the first year. During the second year both numbers on total copper reduced to 0.86 mg/l (Beak 1977). Finally, sediment deposits high in copper and zinc have been observed in operating cooling tower basins (Olin Company 1975).

The actual design of the plant is such that an exact simulation in the laboratory is impossible. During plant operation, pH control is monitored continuously, which was not done in the laboratory. The cooling tower is continuously making up and blowing down water during operation, whereas the laboratory concentration was done as a batch process. Copper tubing used in the condenser system was not simulated in the laboratory experiment. Additions of copper were made post-concentration and ionic copper was used to account for copper as corrosion products. Various other components of the cooling tower (i.e., chlorine and SO_2 injection system) were not simulated either.

Although the laboratory simulation was not exact and there was much variability in the data, the experiments clearly demonstrated the effects of concentrating water on copper toxicity. The bioassays conducted with simulated blowdown water and additions of ionic copper resulted in significantly higher LC_{50} values than those bioassays conducted using ambient river water. Data developed by Samuel Felton (1978) demonstrate that concentrated water has the ability to bind as much as 0.070 to 0.170 mg/l of added ionic copper and therefore make it unavailable to the test

organism. This was also demonstrated in two of the ionic addition bioassays in which chemical analyses showed that for a specified amount of labile copper added to a system, only a fraction of it remained in the labile state. There are many factors involved in the complexing capacity of a given sample of water. Some of these factors are hardness, alkalinity, pH, phosphates, humic acids, suspended solids, and particulates. As a result there is considerable variability in the data. The wide range of LC_{50} values obtained may be due in part to the fact that the bioassays were conducted over the space of a year, and as a result may have tested waters with a varying ability to bind copper.

In view of the results of the bioassays and what is known about the plant design, it is apparent that copper toxicity will not be a problem with respect to the Chehalis River plant. Ambient levels of copper will not be increased enough to cause mortality. Contributions of total copper from corrosion, although large enough to cause potential acute toxicity, will be in the form of insoluble particulates, or if ionic will become complexed so as to make them unavailable to the fish.

SUMMARY

1. Using standard 96-hour bioassays the toxicity of copper and zinc in ambient Chehalis River water and simulated blowdown water to juvenile salmonids was investigated.

2. Fish exposed to solutions of copper and zinc sulfate and ambient river water resulted in 96-hour LC_{50} values from 0.139 mg/1 to 0.242 mg/1 copper, and from 0.094 mg/1 to 0.311 mg/1 zinc.

3. Fish exposed to solutions of copper sulfate and concentrated water resulted in 96-hour LC_{50} values from 0.368 mg/l to 0.611 mg/l copper.

4. The projected theoretical maximum plant discharge of copper is 0.254 mg/l, of which it is estimated that less than 25% will be in the ionic form.

5. In view of rapid dilution and projected loss of copper to the basin of the cooling tower during operation, it can be concluded that copper toxicity will not have a significant effect on juvenile salmonids in the Chehalis River.

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Appendix Tables A1 through A26

Table A1. Results of bioassay no. 1. Test conditions:
(1) Well water, pumped from the vicinity of the plant intake pumps, was concentrated (6x) in a stainless steel container and bioassayed water from Lake Washington and ambient well water was used as controls
(2) Average test temperature: 16.0°C
(3) Ambient concentrations: Cu = 0.003 mg/1, Zn = 0.007 mg/1
(4) Total no. of fish tested: 36
(5) Starting date: 6/6/77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	total %
LW*	0.008	0.002	6	0	0	0	0	0
LW*	0.008	0.002	6	0	0	0	0	0
W+	0.003	0.007	6	0	0	0	0	0
W	0.003	0.007	6	0	0	0	0	0
6x	0.056	0.019	6	0	0	0	0	0
6x	0.056	0.019	6	0	0	0	0	0

* Lake Washington water.
\+ Well water.

Table A2. Results of bioassay no. 2. Test conditions:
(1) Well water pumped from the vicinity of the plant intake pumps, was concentrated (6x) in a stainless steel container and bioassayed. Water from Lake Washington and Chehalis River was used as controls
(2) Average test temperatures: 16.0°C
(3) Ambient concentrations: Cu = 0.003 mg/1, Zn = 0.007 mg/1
(4) Total no. of fish tested: 36
(5) Starting date: 6/6/77
(6) Species tested: coho

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
LW*	0.008	0.002	6	0	0	0	0	0
LW	0.008	0.002	6	0	0	0	0	0
W+	0.003	0.006	6	0	0	0	0	0
W	0.003	0.006	6	0	0	0	0	0
6x	0.056	0.019	6	0	0	0	0	0
6x	0.056	0.019	6	0	0	0	0	0

* Lake Washington water was used as additional control.

+ Well water, pumped from the vicinity of plant intake pumps, was used as test water.

Table A3. Results of bioassay no. 3. Test conditions:
(1) Water from the Chehalis was concentrated (6x). Water from Lake Washington and the Chehalis River was sued as controls.
(2) Average test temperatures: 8.9°C
(3) Ambient Chehalis concentrations: Cu = 0.005 mg/1, Zn = 0.004 mg/1
(4) Total no. fish tested: 36
(5) Starting date: 6/13/77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.005	0.004	6	0	0	0	0	0
Ambient	0.005	0.004	6	0	0	0	0	0
LW*	0.003	0.007	6	0	0	0	0	0
LW	0.003	0.007	6	0	0	0	0	0
6x	0.012	0.013	6	0	0	0	0	0
6x	0.012	0.013	6	0	0	0	0	0

* Water from Lake Washington was used as an additional control.

Table A4. Results of bioassay no. 4. Test conditions:
(1) Water from the Chehalis River was concentrated (6x) and bioassayed water from Lake Washington and the Chehalis River was used as controls
(2) Average test temperatures: 18.9°C
(3) Ambient Chehalis concentrations: Cu = 0.005 mg/1, Zn = 0.004 mg/1
(4) Total no. of fish tested: 36
(5) Starting date: 6/13/77
(6) Species tested: Coho salmon

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.005	0.004	6	0	0	0	0	0
Ambient	0.005	0.004	6	0	0	0	0	0
LW*	0.003	0.007	6	0	0	0	0	0
LW	0.003	0.007	6	0	0	0	0	0
6x	0.012	0.013	6	0	0	0	0	0
6x	0.012	0.013	6	0	0	0	0	0

* Water from Lake Washington used as an additional control.

Table A5. Results of bioassay no. 6. Conditions:
(1) Water from the Chehalis River was concentrated 6 (6x) and 12 (12x) times in a stainless steel container and bioassayed Unconcentrated water from the Chehalis was used as a control
(2) Average test temperature: 19.0°C
(3) Ambient Chehalis concentrations: Cu = 0.004 mg/1, Zn = 0.003 mg/1
(4) Total number of fish tested: 60
(5) Starting date: 7/11/77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/1)	Total Zn (mg/1)	Tank No.	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.004	0.003	1	5	0	0	0	0	0
Ambient	0.004	0.003	2	5	0	0	0	0	0
Ambient	0.004	0.003	3	5	0	0	0	0	0
Ambient	0.004	0.003	4	5	0	0	0	0	0
6x	0.007	0.005	5	5	0	0	0	0	0
6x	0.007	0.005	6	5	0	0	0	0	0
6x	0.007	0.005	7	5	0	0	0	0	0
6x	0.007	0.005	8	5	0	1	1	1	20
12x	0.007	0.002	9	5	0	0	0	0	0
12x	0.007	0.002	10	5	0	0	0	0	0
12x	0.007	0.002	11	5	0	0	0	0	0
12x	0.007	0.002	12	5	0	0	0	0	0

Table A6. Results of bioassay no. 8. Conditions:
(1) Water from the Chehalis River was concentrated 6 (6x) and 12 (12x) times in a stainless steel container and bioassayed. Unconcentrated Chehalis River was used as a control
(2) Average test temperature: 19.5°C
(3) Ambient Chehalis water concentrations: Cu = N.D., Zn = 0.002 mg/l
(4) Total number of fish teste: 60
(5) Starting date: 7/18/77
(6) Species tested: rainbow trout

Test water	Total Cu (mg/l)	Total Zn (mg/l)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	N.D.	0.002	5	0	0	0	0	0
Ambient	N.D.	0.002	5	0	0	0	0	0
Ambient	N.D.	0.002	5	0	0	0	0	0
Ambient	N.D.	0.002	5	0	0	0	0	0
6x	0.009	0.052	5	0	0	0	0	0
6x	0.009	0.052	5	0	0	0	0	0
6x	0.009	0.052	5	0	0	0	0	0
12x	0.015	0.063	5	0	0	0	0	0
12x	0.015	0.063	5	0	0	0	0	0
12x	0.015	0.063	5	0	0	0	0	0
12x	0.015	0.063	5	0	0	0	0	0

Table A7. Results of bioassay no. 14. Conditions:
(1) Water from the Chehalis River was concentrated (6x, 12x) in a stainless steel container and bioassayed. Unconcentrated Chehalis water was used as a control
(2) Average test temperature: 12.2°C
(3) Ambient Chehalis water concentrations: Cu = 0.003 mg/1, Zn = 0.008 mg/1
(4) Total number of fish tested: 80
(5) Starting date: 11-1-77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.003	0.008	10	0	0	0	0	0
Ambient	0.003	0.008	10	0	0	0	0	0
6x	0.015	0.008	10	0	0	0	0	0
6x	0.015	0.008	10	0	0	0	0	0
6x	0.015	0.008	10	0	0	0	0	0
12x	0.019	0.032	10	0	0	0	0	0
12x	0.019	0.032	10	0	0	0	0	0
12x	0.019	0.032	10	0	0	0	0	0

Table A8. Results of bioassay no. 15. Conditions:
(1) Water from the Chehalis River was concentrated (6x, 12x) in a stainless steel container and bioassayed. Unconcentrated Chehalis water was used as a control.
(2) Average test temperature: 10°C
(3) Ambient Chehalis water concentrations: Cu = N.D., Zn = 0.005 mg/1
(4) Total number of fish tested: 80
(5) Starting date: 11-15-77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	N.D.	0.005	10	0	0	0	0	0
Ambient	N.D.	0.005	10	0	0	0	0	0
6x	0.016	0.008	10	0	0	0	0	0
6x	0.016	0.008	10	0	0	0	0	0
12x	0.011	0.032	10	0	0	0	0	0
12x	0.011	0.032	10	0	0	0	0	0
12x	0.011	0.032	10	0	0	0	0	0

Table A9. Results of bioassay no. 16. Conditions:
(1) Chehalis River water was spiked with copper and zinc, then concentrated (6x, 12x, 18x) in a stainless steel container for bioassay. Ambient Chehalis R. water used as control.
(2) Average test temperature: 9.9°C
(3) Ambient Chehalis concentrations: Cu = 0.003 mg/1, Zn = 0.005 mg/1
Copper and zinc levels after spiking and before concentration: Cu = 27 ppb, Zn = 40 ppb
(4) Total number of fish tested: 140
(5) Starting date: 12-2-77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.003	0.005	10	0	0	0	0	0
Ambient	0.003	0.005	10	0	0	0	0	0
Spiked ambient	0.027	0.040	10	0	0	0	0	0
Spiked ambient	0.027	0.040	10	0	0	0	0	0
Spiked ambient	0.027	0.040	10	0	0	0	0	0
6x	0.073	0.054	10	0	0	0	0	0
6x	0.073	0.054	10	0	0	0	0	0
6x	0.073	0.054	10	0	0	0	0	0
12x	0.133	0.083	10	0	0	0	0	0
12x	0.133	0.083	10	0	0	0	0	0
12x	0.133	0.083	10	0	0	0	0	0
18x	0.162	0.104	10	0	0	0	0	0
18x	0.162	0.104	10	0	0	0	0	0
18x	0.162	0.104	10	0	0	0	0	0

Table A10. Results of test 5. Conditions:
(1) Copper sulfate was added to ambient Chehalis River water at indicated levels for bioassay. Water from the Chehalis River was used as a control.
(2) Average test temperature: 19.5°C
(3) Ambient Chehalis concentrations: Cu = 0.007 mg/l, Zn = 0.004 mg/l
(4) Total number of fish tested = 75
(5) Starting date: 6-27-77
(6) Test species tested: Coho

Tank water	Total Cu (mg/l)	Tank no.	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.007	1	5	0	0	0	0	20
Ambient	0.007	2	5	0	0	0	0	0
Ambient	0.007	3	5	0	0	0	0	0
Ambient + 0.015 mg/l	0.022	4	5	0	0	0	1	20
Ambient + 0.015 mg/l	0.022	5	5	0	0	3	3	60
Ambient + 0.015 mg/l	0.022	6	5	0	0	0	0	0
Ambient + 0.037 mg/l	0.044	7	5	0	0	0	0	0
Ambient + 0.037 mg/l	0.044	8	5	0	0	0	0	0
Ambient + 0.037 mg/l	0.044	9	5	0	0	0	0	0
Ambient + 0.082 mg/l	0.089	10	5	0	0	2	2	40
Ambient + 0.082 mg/l	0.089	11	5	0	0	0	0	0
Ambient + 0.082 mg/l	0.089	12	5	0	1	1	1	20
Ambient + 0.0126 mg/l	0.133	13	5	0	1	1	1	20
Ambient + 0.0126 mg/l	0.133	14	5	2	3	3	3	60
Ambient + 0.0126 mg/l	0.133	15	5	5	5	5	5	100

Table A11. Results of bioassay no. 10. Test conditions:
(1) Copper sulfate was added to ambient Chehalis River water at indicated levels for bioassay. Chehalis River water was used as a control.
(2) Average test temperature: 21.5°C
(3) Ambient Chehalis concentration: Cu = 0.002 mg/1, Zn = N.D.
(4) Total number of fish tested = 75
(5) Starting date: 8-1-77
(6) Test species: Coho salmon

Test water	Total Cu (mg/1)	No. fish per tank	Cumulative mortalities				Total %
			24 hr	48 hr	72 hr	96 hr	
Ambient	0.002	5	0	0	0	0	0
Ambient	0.002	5	0	0	0	0	0
Ambient	0.002	5	0	0	0	0	0
Ambient + 0.007 mg/1	0.009	5	0	0	0	0	0
Ambient + 0.007 mg/1	0.009	5	0	0	0	0	0
Ambient + 0.007 mg/1	0.009	5	0	0	0	0	0
Ambient + 0.016 mg/1	0.018	5	0	0	0	0	0
Ambient + 0.016 mg/1	0.018	5	0	0	0	0	0
Ambient + 0.016 mg/1	0.018	5	0	0	0	0	0
Ambient + 0.025 mg/1	0.027	5	0	0	0	0	0
Ambient + 0.025 mg/1	0.027	5	0	0	0	0	0
Ambient + 0.025 mg/1	0.027	5	0	0	0	0	0
Ambient + 0.034 mg/1	0.036	5	0	0	0	0	0
Ambient + 0.034 mg/1	0.036	5	0	0	0	0	0
Ambient + 0.034 mg/1	0.036	5	0	0	0	0	0

Table A12. Results test 12. Test conditions:
(1) Copper sulfate was added to Chehalis River water at indicated levels for bioassay. Water from the Chehalis River was used as a control.
(2) Average test temperature: 12.5°C
(3) Ambient Chehalis water concentrations: Cu = 0.003 mg/l Zn = 0.008 mg/l
(4) Total number of fish tested: 120
(5) Starting date: 10-31-77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/l)	No. fish per tank	Cumulative mortalities				Total %
			24 hr	48 hr	72 hr	96 hr	
Ambient	0.003	10	0	0	0	0	0
Ambient	0.003	10	0	0	0	0	0
Ambient + 0.006 mg/l	0.009	10	0	0	0	0	0
Ambient + 0.006 mg/l	0.009	10	0	0	0	0	0
Ambient + 0.015 mg/l	0.018	10	0	0	0	0	0
Ambient + 0.033 mg/l	0.036	10	0	0	0	0	0
Ambient + 0.033 mg/l	0.036	10	0	0	0	0	0
Ambient + 0.053 mg/l	0.056	10	0	0	0	0	0
Ambient + 0.053 mg/l	0.056	10	0	0	0	0	0
Ambient + 0.069 mg/l	0.072	10	0	0	0	0	0
Ambient + 0.069 mg/l	0.072	10	0	0	0	0	0

Table A13. Results of test no. 17. Conditions:
(1) Copper sulfate was added to Chehalis River water at indicated levels for bioassay. Unconcentrated Chehalis River water was used as a control.
(2) Average test temperature: 9.5°C
(3) Ambient Chehalis water concentrations: Cu = 0.003 mg/l
Zn = 0.005 mg/l
(4) Total no. of fish tested: 120
(5) Starting date: 12-5-77
(6) Species tested: Rainbow trout

Test water	Total Cu (mg/l)	No. fish per tank	Cumulative mortalities				Total %
			24 hr	48 hr	72 hr	96 hr	
Ambient	0.003	10	0	0	0	0	0
Ambient	0.003	10	0	0	0	0	0
Ambient + 0.047 mg/l	0.050	10	0	0	0	0	0
Ambient + 0.047 mg/l	0.050	10	0	0	0	0	0
Ambient + 0.097 mg/l	0.100	10	0	0	2	2	20
Ambient + 0.097 mg/l	0.100	10	0	0	2	2	20
Ambient + 0.197 mg/l	0.200	10	0	1	1	2	20
Ambient + 0.197 mg/l	0.200	10	0	0	0	0	0
Ambient + 0.297 mg/l	0.300	10	0	6	6	6	60
Ambient + 0.297 mg/l	0.300	10	0	3	7	7	70
Ambient + 0.397 mg/l	0.400	10	1	10	10	10	100
Ambient + 0.397 mg/l	0.400	10	1	7	8	8	80

Table A14. Results of test no. 19. Conditions:
(1) Copper sulfate was added to Chehalis River water at indicated levels for bioassay. Unconcentrated Chehalis River water was used as a control.
(2) Average test temperature: 7.9°C
(3) Ambient Chehalis water concentrations: Cu = 0.005 mg/l
Zn = 0.006 mg/l
(4) Total number of fish tested: 120
(5) Starting date: 1-16-78
(6) Species tested: chinook salmon

Test water	Total Cu (mg/l)	No. fish per tank	Cumulative mortalities				Total %
			24 hr	48 hr	72 hr	96 hr	
Ambient	0.005	10	0	0	0	0	0
Ambient	0.005	10	0	0	0	0	0
Ambient + 0.045 mg/l	0.050	10	0	0	0	0	0
Ambient + 0.045 mg/l	0.050	10	0	0	0	0	0
Ambient + 0.095 mg/l	0.100	10	0	0	0	0	0
Ambient + 0.095 mg/l	0.100	10	0	0	0	0	0
Ambient + 0.195 mg/l	0.200	10	0	1	4	6	60
Ambient + 0.195 mg/l	0.200	10	0	1	3	3	30
Ambient + 0.295 mg/l	0.300	10	0	10	10	10	100
Ambient + 0.295 mg/l	0.300	10	0	10	10	10	100
Ambient + 0.395 mg/l	0.400	10	0	10	10	10	100
Ambient + 0.395 mg/l	0.400	10	0	10	10	10	100

Table A15. Results of test no. 20. Conditions:
(1) Zinc sulfate was added to Chehalis River water at indicated levels for bioassay. Unconcentrated river water was used as a control.
(2) Average test temperature: 7.9°C
(3) Ambient Chehalis water concentrations: Cu = 0.005 mg/l Zn = 0.006 mg/l
(4) Total number of fish tested: 120
(5) Starting date: 1-16-78
(6) Species tested: chinook salmon

Test water	Total Zn (mg/l)	No. fish per tank	Cumulative mortalities				Total %
			24 hr	48 hr	72 hr	96 hr	
Ambient	0.006	10	0	0	0	0	0
Ambient	0.006	10	0	0	0	0	0
Ambient + 0.095 mg/l	0.100	10	0	0	3	7	70
Ambient + 0.095 mg/l	0.100	10	0	0	3	5	50
Ambient + 0.195 mg/l	0.200	10	0	6	10	10	100
Ambient + 0.195 mg/l	0.200	10	0	7	9	9	90
Ambient + 0.495 mg/l	0.500	10	0	9	10	10	100
Ambient + 0.495 mg/l	0.500	10	0	10	10	10	100
Ambient + 0.995 mg/l	1.000	10	0	10	10	10	100
Ambient + 0.995 mg/l	1.000	10	0	10	10	10	100
Ambient + 1.245 mg/l	1.250	10	0	7	10	10	100
Ambient + 1.245 mg/l	1.250	10	0	10	10	10	100

Table A16. Results of test 7. Conditions:
(1) Zinc sulfate was added to ambient Chehalis River water at indicated levels for bioassay. Water from the Chehalis River was used as a control.
(2) Average test temperature: 19.1°C
(3) Ambient Chehalis concentrations: Zn = 0.005 mg/1, Cu = 0.004 mg/1
(4) Total number of fish tested = 90
(5) Starting date: 7-11-77
(6) Species tested: Rainbow trout

Test water	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total o %
Ambient	0.005	6	0	0	0	0	0
Ambient	0.005	6	0	0	0	0	0
Ambient	0.005	6	**	**	**	**	**
Ambient + 0.011 mg/1	0.016	6	0	0	0	0	0
Ambient + 0.011 mg/1	0.016	6	0	0	0	0	0
Ambient + 0.011 mg/1	0.016	6	0	0	0	0	0
Ambient + 0.027 mg/1	0.032	6	**	**	**	**	**
Ambient + 0.027 mg/1	0.032	6	0	0	0	0	0
Ambient + 0.027 mg/1	0.032	6	0	0	0	0	0
Ambient + 0.059 mg/1	0.064	6	**	**	**	**	**
Ambient + 0.059 mg/1	0.064	6	**	**	**	**	**
Ambient + 0.059 mg/1	0.064	6	0	0	0	0	0
Ambient + 0.090 mg/1	0.095	6	0	0	0	0	0
Ambient + 0.090 mg/1	0.095	6	0	0	0	0	0
Ambient + 0.090 mg/1	0.095	6	**	**	**	**	**

**The observation was discarded due to aerator failure and resultant low D.O.

Table A17. Results of bioassay no. 9. Test conditions:
(1) Zinc sulfate was added to ambient Chehalis River water at indicated levels for bioassay. Water from the Chehalis River was used as control.
(2) Average test temperatures: 20.0°C
(3) Ambient Chehalis concentrations: Cu = N.D., Zn = 0.009 mg/1
(4) Total number of fish tested = 75
(5) Starting date: 7-19-77
(6) Test species: Rainbow trout

Test water	Total Zn (mg/1)	no. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.009	5	0	0	0	0	0
Ambient	0.009	5	0	0	0	0	0
Ambient	0.026	5	0	0	0	0	0
Ambient + 0.017 mg/1	0.026	5	0	0	0	**	**
Ambient + 0.017 mg/1	0.026	5	0	0	0	0	0
Ambient + 0.017 mg/1	0.051	5	0	0	0	0	0
Ambient + 0.042 mg/1	0.051	5	0	0	0	0	0
Ambient + 0.042 mg/1	0.051	5	1	1	1	1	10
Ambient + 0.042 mg/1	0.102	5	0	0	0	**	**
Ambient + 0.093 mg/1	0.102	5	0	0	0	0	0
Ambient + 0.093 mg/1	0.102	5	0	0	0	0	0
Ambient + 0.143 mg/1	0.153	5	0	0	0	0	0
Ambient + 0.143 mg/1	0.153	5	0	0	0	0	0
Ambient + 0.143 mg/1	0.153	5	0	0	0	0	0

**Observation was discarded due to aerator failure and resultant low D.O.

Table A18. Results of test 11. Test conditions:
(1) Zinc sulfate was added to ambient Chehalis River water at indicated levels for bioassay. Water from the Chehalis River was used as control.
(2) Average test temperatures: 24°C
(3) Ambient Chehalis concentrations: Cu = N.A., Zn = 0.009 mg/1
(4) Total number of fish tested = 60
(5) Starting date: 8-9-77
(6) Test species: Coho salmon

Test water	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.009	5	**	**	**	**	**
Ambient	0.009	5	**	**	**	**	**
Ambient	0.009	5	0	0	0	0	0
Ambient + 0.144 mg/1	0.153	5	0	0	0	0	0
Ambient + 0.144 mg/1	0.153	5	0	0	0	0	0
Ambient + 0.144 mg/1	0.153	5	0	0	0	0	0
Ambient + 0.246 mg/1	0.255	5	0	0	0	0	0
Ambient + 0.246 mg/1	0.255	5	0	0	0	0	0
Ambient + 0.246 mg/1	0.255	5	0	0	0	0	0
Ambient + 0.841 mg/1	0.850	5	0	0	2	4	80
Ambient + 0.841 mg/1	0.850	5	1	1	3	4	80
Ambient + 0.841 mg/1	0.850	5	0	0	1	2	40

**Observation was discarded due to aerator failure and resultant low D.O.

Table A19. Results of test no. 13. Conditions:
(1) Zinc sulfate was added to Chehalis River water of indicated levels for bioassay. Water from the Chehalis River was used as a control.
(2) Average test temperature: 12.5°C
(3) Ambient Chehalis water concentrations: Cu = 0.003 mg/l Zn = 0.008 mg/l
(4) Total number of fish tested: 120
(5) Starting date: 10-31-77
(6) Species tested: Rainbow trout

Test water	Total Zn (mg/l)	No. fish per tank	Cumulative mortalities				Total %
			24 hr	48 hr	72 hr	96 hr	
Ambient	0.008	10	0	0	0	0	0
Ambient	0.008	10	0	0	0	0	0
Ambient + 0.016 mg/l	0.024	10	0	0	0	0	0
Ambient + 0.016 mg/l	0.024	10	0	1	1	1	10
Ambient + 0.040 mg/l	0.048	10	0	0	0	0	0
Ambient + 0.040 mg/l	0.048	10	0	0	0	0	0
Ambient + 0.088 mg/l	0.096	10	0	0	0	0	0
Ambient + 0.088 mg/l	0.096	10	0	0	1	1	10
Ambient + 0.136 mg/l	0.144	10	0	1	3	3	30
Ambient + 0.136 mg/l	0.144	10	0	1	2	2	20
Ambient + 0.184 mg/l	0.192	10	0	2	6	6	60
Ambient + 0.184 mg/l	0.192	10	0	1	2	2	20

Table A20. Results of test no. 18. Conditions:
(1) Zinc sulfate was added to Chehalis River water at indicated levels for bioassay. Unconcentrated Chehalis River water was used as a control.
(2) Average test temperature: 9.5°C
(3) Ambient Chehalis water concentrations: Cu = 0.003 mg/l
Zn = 0.005 mg/l
(4) Total number of fish tested: 140
(5) Starting date: 12-5-77

Test water	Total Zn (mg/l)	No. fish per tank	Cumulative mortalities				Total %
			24 hr	48 hr	72 hr	96 hr	
Ambient	0.005	10	0	0	0	0	0
Ambient	0.005	10	0	0	0	0	0
Ambient + 0.095 mg/l	0.100	10	0	0	1	2	20
Ambient + 0.095 mg/l	0.100	10	0	0	3	4	40
Ambient + 0.245 mg/l	0.250	10	0	0	1	7	70
Ambient + 0.245 mg/l	0.250	10	0	2	4	5	50
Ambient + 0.495 mg/l	0.500	10	0	0	8	9	90
Ambient + 0.495 mg/l	0.500	10	0	2	3	7	70
Ambient + 0.745 mg/l	0.750	10	0	0	4	9	90
Ambient + 0.745 mg/l	0.750	10	0	1	5	7	70
Ambient + 0.995 mg/l	1.000	10	0	0	3	6	60
Ambient + 0.995 mg/l	1.000	10	0	1	3	6	60
Ambient + 1.245 mg/l	1.250	10	0	3	5	7	70
Ambient + 1.245 mg/l	1.250	10	0	2	6	6	60

Table A21. Results of test no. 21. Conditions:
(1) Copper and zinc sulfate were added to Chehalis River water at indicated levels and this water was bioassayed. Unconcentrated Chehalis River water was used as a control.
(2) Average test temperature: 7.5°C
(3) Ambient Chehalis water concentrations: Cu = 0.008 mg/1, Zn = 0.005 mg/1
(4) Total number of fish tested: 120
(5) Starting date: 1-23-78
(6) Test species: Chinook salmon

Test water	Total Cu (mg/1)	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.008	0.005	10	0	0	0	0	0
Ambient	0.008	0.005	10	0	0	0	0	0
Ambient + 0.015 mg/1 Cu, 0.010 mg/1 Zn	0.023	0.015	10	0	0	0	0	0
Ambient + 0.015 mg/1 Cu, 0.010 mg/1 Zn	0.023	0.015	10	0	0	0	0	0
Ambient + 0.037 mg/1 Cu, 0.025 mg/1 Zn	0.045	0.030	10	5	10	10	10	100
Ambient + 0.037 mg/1 Cu, 0.025 mg/1 Zn	0.045	0.030	10	0	0	0	0	0
Ambient + 0.082 mg/1 Cu, 0.055 mg/1 Zn	0.090	0.060	10	0	0	0	0	0
Ambient + 0.082 mg/1 Cu, 0.055 mg/1 Zn	0.090	0.060	10	0	0	0	0	0
Ambient + 0.217 mg/1 Cu, 0.145 mg/1 Zn	0.225	0.150	10	0	8	10	10	100
Ambient + 0.217 mg/1 Cu, 0.145 mg/1 Zn	0.225	0.150	10	2	8	10	10	100
Ambient + 0.367 mg/1 Cu, 0.245 mg/1 Zn	0.375	0.250	10	3	4	10	10	100
Ambient + 0.367 mg/1 Cu, 0.245 mg/1 Zn	0.375	0.250	10	3	5	10	10	100

Table A22. Results of bioassay no. 22. Conditions:
(1) Copper and zinc sulfate were added to test containers at indicated levels for bioassay.
(2) Average test temperature: 7.8°C
(3) Ambient Chehalis water concentrations: Cu = 0.010 mg/l, Zn = 0.006 mg/l
(4) Total number of fish tested: 260
(5) Starting date: 2-14-78
(6) Species tested: Chinook salmon

Test water	Total Cu (mg/l)	Total Zn (mg/l)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	0.010	0.006	10	0	0	0	0	0
Ambient	0.010	0.006	10	0	0	0	0	0
Ambient + 0.050 mg/l Cu	0.060	0.006	10	0	0	0	2	20
Ambient + 0.050 mg/l Cu	0.060	0.006	10	0	0	1	2	20
Ambient + 0.110 mg/l Cu	0.120	0.006	10	0	0	3	8	80
Ambient + 0.110 mg/l Cu	0.120	0.006	10	0	0	0	0	0
Ambient + 0.230 mg/l Cu	0.240	0.006	10	0	9	10	10	100
Ambient + 0.230 mg/l Cu	0.240	0.006	10	0	10	10	10	100
Ambient + 0.027 mg/l Zn	0.010	0.033	10	0	0	0	0	0
Ambient + 0.027 mg/l Zn	0.010	0.033	10	0	0	0	0	0
Ambient + 0.060 mg/l Zn	0.010	0.066	10	0	0	0	0	0
Ambient + 0.060 mg/l Zn	0.010	0.066	10	0	0	0	0	0
Ambient + 0.126 mg/l Zn	0.010	0.132	10	0	0	1	1	10
Ambient + 0.126 mg/l Zn	0.010	0.132	10	0	1	1	1	10
Ambient + 0.170 mg/l Zn	0.010	0.176	10	0	3	7	10	100
Ambient + 0.170 mg/l Zn	0.010	0.176	10	0	4	8	10	100
Ambient + 0.020 mg/l Cu, 0.011 mg/l Zn	0.030	0.017	10	0	0	0	0	0
Ambient + 0.020 mg/l Cu, 0.011 mg/l Zn	0.030	0.017	10	0	0	0	2	0
Ambient + 0.050 mg/l Cu, 0.011 mg/l Zn	0.060	0.033	10	0	0	0	1	20
Ambient + 0.050 mg/l Cu, 0.011 mg/l Zn	0.060	0.033	10	0	0	9	9	10
Ambient + 0.110 mg/l Cu, 0.060 mg/l Zn	0.120	0.066	10	0	0	6	8	90
Ambient + 0.110 mg/l Cu, 0.060 mg/l Zn	0.120	0.066	10	0	0	10	10	80
Ambient + 0.220 mg/l Cu, 0.126 mg/l Zn	0.240	0.132	10	0	10	10	10	100
Ambient + 0.220 mg/l Cu, 0.126 mg/l Zn	0.240	0.132	10	0	10	6	9	100
Ambient + 0.350 mg/l Cu, 0.192 mg/l Zn	0.360	0.198	10	0	3	6	9	90
Ambient + 0.350 mg/l Cu, 0.192 mg/l Zn	0.360	0.198	10	0	1	6	9	90

Table A23. Results of bioassay no. 23. Test conditions:

(1) Chehalis River water was spiked with copper and zinc sulfates and concentrated (6x). After concentration further addition of copper sulfate was made at the indicated levels. The water was then bioassayed. Test solutions was adjusted to pH of 7.0.
(2) Average test temperature: 14.6°C
(3) Ambient Chehalis concentrations: Cu = 0.006 mg/l, Zn = 0.008 mg/l
(4) Total number of fish tested: 180
(5) Starting date: 5-26-78
(6) Species tested: Rainbow trout

Test water	pH	Total Cu (mg/l)	Total Zn (mg/l)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	7.2	0.006	0.008	10	0	0	1	1	10
Ambient	7.2	0.006	0.008	10	0	0	0	0	0
Ambient spiked	7.2	0.059	0.062	10	0	1	1	1	10
Ambient spiked	7.2	0.059	0.062	10	0	3	4	4	40
6x	7.0	0.158	0.084	10	0	0	0	0	0
6x	7.0	0.158	0.084	10	0	0	0	0	0
6x + 0.400 mg/l Cu	7.0	0.558	0.084	10	3	7	7	7	70
6x + 0.400 mg/l Cu	7.0	0.558	0.084	10	0	0	0	0	0
6x + 0.500 mg/l Cu	7.0	0.658	0.084	10	2	6	7	7	70
6x + 0.500 mg/l Cu	7.0	0.658	0.084	10	1	6	6	6	60
6x + 0.500 mg/l Cu	7.0	0.758	0.084	10	0	7	7	7	70
6x + 0.600 mg/l Cu	7.0	0.758	0.084	10	0	6	6	6	60
6x + 0.700 mg/l Cu	7.0	0.858	0.084	10	6	10	10	10	100
6x + 0.700 mg/l Cu	7.0	0.858	0.084	10	4	10	10	10	100
6x + 0.800 mg/l Cu	7.0	0.958	0.084	10	8	10	10	10	100
6x + 0.800 mg/l Cu	7.0	0.958	0.084	10	6	9	9	9	90
6x + 1.000 mg/l Cu	7.0	1.158	0.084	10	10	10	10	10	100
6x + 1.000 mg/l Cu	7.0	1.158	0.084	10	9	9	9	9	90

Table A24. Results of bioassay no. 24. Test conditions:

(1) Chehalis River water was spiked with copper and zinc sulfate and concentrated (6x). After concentration further additions of copper sulfate were made at the indicated levels. The water was then bioassayed. Test solutions were adjusted to pH of 7.0.
(2) Average test temperature: 19.8°C
(3) Ambient Chehalis concentrations: Cu = 0.005 mg/1, Zn = 0.004 mg/1
(4) Total number of fish tested: 200
(5) Starting date: 6-5-78
(6) Species tested: Rainbow trout

Test water	pH	Total copper (mg/1)	Labile copper (mg/1)	Total zinc (mg/1)	Labile zinc (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	7.0	0.005	N.D.[a]	0.004	N.D.	10	0	0	0	0	0
Ambient	7.0	0.005	N.D.	0.004	N.D.	10	0	0	0	0	0
Spiked Ambient	7.0	0.013	N.A.[b]	0.045	N.A.	10	0	0	0	0	0
Spiked Ambient	7.0	0.013	N.A.	0.045	N.A.	10	0	0	0	0	0
6x	7.0	0.079	N.A.	0.101	N.A.	10	0	0	0	0	0
6x	7.0	0.079	N.A.	0.101	N.A.	10	0	0	0	0	0
6x + 0.200 mg/1 Cu	7.0	0.279	0.059	0.101	0.021	10	2	9	9	9	90
6x + 0.200 mg/1 Cu	7.0	0.279	0.051	0.101	0.018	10	3	9	9	9	90
6x + 0.300 mg/1 Cu	7.0	0.379	0.118	0.101	0.033	10	1	10	10	10	100
6x + 0.300 mg/1 Cu	7.0	0.379	0.107	0.101	0.029	10	2	8	8	8	80
6x + 0.400 mg/1 Cu	7.0	0.479	0.134	0.101	0.026	10	1	6	6	6	60
6x + 0.400 mg/1 Cu	7.0	0.479	0.142	0.101	0.032	10	10	10	10	10	100
6x + 0.500 mg/1 Cu	7.0	0.579	0.165	0.101	0.014	10	3	9	9	9	90
6x + 0.500 mg/1 Cu	7.0	0.579	0.148	0.101	0.026	10	5	9	9	9	90
6x + 0.600 mg/1 Cu	7.0	0.679	0.215	0.101	0.012	10	2	10	10	10	100
6x + 0.600 mg/1 Cu	7.0	0.679	0.348	0.101	0.049	10	4	8	8	8	80
6x + 0.700 mg/1 Cu	7.0	0.779	0.434	0.101	0.064	10	5	10	10	10	100
6x + 0.700 mg/1 Cu	7.0	0.779	0.250	0.101	0.047	10	5	9	9	9	90
6x + 0.800 mg/1 Cu	7.0	0.889	0.382	0.101	0.056	10	1	6	8	8	80
6x + 0.800 mg/1 Cu	7.0	0.889	N.A.	0.101	-	10	5	8	8	8	80

a. N.D. means none detectable.
b. N.A. means not available.

Table A25. Results of bioassay no. 25. Test conditions:
(1) Chehalis River water was concentrated (6x). After concentrations copper sulfate was added at indicated levels and the water was bioassayed. Test solutions were adjusted to levels of pH 7.0.
(2) Average test temperature: 17.4°C
(3) Ambient Chehalis concentrations: Cu N.D., Zn = 0.007 mg/1
(4) Total number of fish tested: 160
(5) Starting date: 6-15-78
(6) Species tested: Rainbow trout

Test water	pH	Total copper (mg/1)	Ionic copper (mg/1)	Total zinc (mg/1)	Ionic zinc (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Ambient	7.3	N.D.	N.D.	0.007	N.D.	10	0	0	0	0	0
Ambient	7.3	N.D.	N.D.	0.007	N.D.	10	0	0	0	0	0
6x	7.0	N.D.	N.D.	0.021	0.003	10	0	0	0	0	0
6x	7.0	N.D.	N.D.	0.021	0.003	10	0	0	0	0	0
6x + 0.050 mg/1 Cu	7.0	0.050	N.D.	0.021	N.D.	10	0	0	0	0	0
6x + 0.050 mg/1 Cu	7.0	0.050	0.014	0.021	0.002	10	0	0	0	0	0
6x + 0.100 mg/1 Cu	7.0	0.100	0.026	0.021	0.003	10	0	0	0	0	0
6x + 0.100 mg/1 Cu	7.0	0.100	N.D.	0.021	0.002	10	0	0	0	0	0
6x + 0.200 mg/1 Cu	7.0	0.200	0.052	0.021	0.003	10	0	0	0	0	0
6x + 0.200 mg/1 Cu	7.0	0.200	0.063	0.021	0.003	10	0	0	0	0	0
6x + 0.400 mg/1 Cu	7.0	0.400	0.083	0.021	0.003	10	4	9	9	9	90
6x + 0.400 mg/1 Cu	7.0	0.400	0.091	0.021	0.003	10	0	4	4	4	40
6x + 0.600 mg/1 Cu	7.0	0.600	0.140	0.021	0.003	10	0	8	9	9	90
6x + 0.600 mg/1 Cu	7.0	0.600	0.370	0.021	N.D.	10	1	10	10	10	100
6x + 0.800 mg/1 Cu	7.0	0.800	0.142	0.021	0.002	10	8	10	10	10	100
6x + 0.800 mg/1 Cu	7.0	0.800	0.335	0.021	N.D.	10	6	8	8	8	80

Table A26. Results of bioassay no. 26. Test conditions:
(1) Chehalis River water was spiked with copper sulfate and concentrated (6x). Additional copper sulfate was added after concentration at levels indicated below and then bioassayed. Test solutions were adjusted to pH of 7.0
(2) Average test temperature: 17.4°C
(3) Ambient Chehalis concentrations: Cu = N.D., Zn = 0.006 mg/1
(4) Total number of fish tested: 180
(5) Starting date: 6-27-78
(6) Species tested: Rainbow trout

Species	Test water	pH	Total Cu (mg/1)	Total Zn (mg/1)	No. fish per tank	Cumulative mortalities 24 hr	48 hr	72 hr	96 hr	Total %
Rainbow	Ambient	7.2	N.D.	0.006	10	0	0	0	0	0
	Ambient	7.2	N.D.	0.006	10	0	*	*	*	*
	Ambient spiked	7.2	0.045	0.011	10	2	8	10	10	100
	Ambient spiked	7.2	0.045	0.011	10	0	1	1	2	20
	6x	7.0	0.111	0.012	10	0	0	0	0	0
	6x	7.0	0.111	0.012	10	0	0	0	1	10
	6x + 0.050 mg/1 Cu	7.0	0.161	0.012	10	0	0	0	0	0
	6x + 0.050 mg/1 Cu	7.0	0.161	0.012	10	0	0	0	*	*
	6x + 0.100 mg/1 Cu	7.0	0.211	0.012	10	0	0	0	0	0
	6x + 0.100 mg/1 Cu	7.0	0.211	0.012	10	0	0	0	0	0
	6x + 0.200 mg/1 Cu	7.0	0.311	0.012	10	0	2	2	4	40
	6x + 0.200 mg/1 Cu	7.0	0.311	0.012	0	0	0	0	0	0
	6x + 0.400 mg/1 Cu	7.0	0.511	0.012	10	5	9	9	9	90
	6x + 0.400 mg/1 Cu	7.0	0.511	0.012	10	5	10	10	10	100
	6x + 0.600 mg/1 Cu	7.0	0.711	0.012	10	8	10	10	10	100
	6x + 0.600 mg/1 Cu	7.0	0.711	0.012	10	6	10	10	10	100
	6x + 0.800 mg/1 Cu	7.0	0.911	0.012	10	10	10	10	10	100
	6x + 0.800 mg/1 Cu	7.0	0.911	0.012	10	7	10	10	10	100

*Observation discarded due to loss of aeration and resultant low D.O.