# Short-Term Exposure of Rainbow Trout (Salmo gairdneri) to an Algicidal Level of Copper Sulphate in Two Hard-Water Ponds

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SHORT-TERM EXPOSURE OF RAINBOW TROUT (*Salmo gairdneri*) TO AN ALGICIDAL LEVEL OF COPPER SULPHATE IN TWO HARD-WATER PONDS

by

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This is the second manuscript report from the Western Region, Winnipeg



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#### ABSTRACT

#### Majewski, H.S., R.W. Danell, J. Barica, A. Lutz, and J.F. Klaverkamp. 1978. Short-term exposure of rainbow trout (*Salmo gairdneri*) to an algicidal level of copper sulphate in two hard-water ponds. Can. Fish. Mar. Serv. MS Rep. 1448: v + 10 p.

Rainbow trout (Salmo gairdneri) were stocked into two prairie pothole lakes, and exposed for 10 days to a concentration of  $CuSO_4$ effective for the control of algae. Each lake was partitioned, such that one side was treated with 0.5 mg/L  $CuSO_4.5H_2O$  (0.125 mg/L Cu), and the other served as the control. Initial concentrations of total and ionic Cu in the water were reduced to about 50% within two-three days after application, and then decreased to trace levels within 10 days. Liver and kidney [Cu] did not change significantly over this period of time. However, a strong correlation between liver [Cu] and fish weight was found. Some possible consequences of continued CuSO<sub>4</sub> treatment of ponds are discussed, as well as points to consider when choosing the proper [Cu] for effective algal control.

Key words: aquaculture; copper; algicides; liver; kidney; accumulation.

#### RESUME

Majewski, H.S., R.W. Danell, J. Barica, A. Lutz, and J.F. Klaverkamp. 1978. Short-term exposure of rainbow trout (*Salmo gairdneri*) to an algicidal level of copper sulphate in two hard-water ponds. Can. Fish. Mar. Serv. MS Rep. 1448: v + 10 p.

Des truites arc-en-ciel (*Salmo gairdneri*) ont été placées dans deux étangs naturels (potholes) des prairies pour y être exposées, pendant dix jours, à une concentration algicide du CuSO<sub>4</sub>. Chaque étang a été divisée en deux: une moitié a été traitée au CuSO<sub>4</sub>.5H<sub>2</sub>O à raison de 0.5 mg/L (0.125 mg/L Cu), et l'autre a servi de témoin. La concentration totale de cuivre et d'ions de cuivre a diminué d'environ 50% après deux ou trois jours. Au bout de dix jours, le cuivre a été reduit à l'état de traces. Durant cette période, la concentration de [Cu] dans les reins et le foie des truites ne s'est pas modifiée de façon sensible. On a toutefois établi une forte corrélation entre [Cu] dans le foie et le poids de l'animal. Le rapport commente les conséquences possibles de la poursuite du traitement au CuSO<sub>4</sub> dans les étangs, de même que les aspects à considérer dans le choix de la [Cu] léthale pour les algues.

Mots-cles: aquaculture; cuivre; algicides; foie; rein; accumulation.

#### INTRODUCTION

Copper sulphate (CuSO<sub>4</sub>) has been used extensively for the control of algal blooms (usually *Aphanizomenon flos-aquae*) by the trout farming industry in Central Canada (Whitaker et al. 1977). The sudden collapse of these blooms with resultant decomposition of dead algal cells, and subsequent oxygen depletion (summerkill), is considered to be one of the major factors adversely affecting rainbow trout survival in Canadian pothole lakes (Lawler et al. 1974; Barica 1975; Ayles et al. 1976). It is not known, however, if the concentrations of CuSO<sub>4</sub> used for algal control in these lakes result in accumulation of Cu in trout.

Life (1970) demonstrated that goldfish (*Carassius auratus*) lacked metabolic mechanisms to dispose of accumulated Cu. Within 24 hours after caudal artery injection, these fish accumulated 65.3%  $^{64}Cu^{2+}$  in the liver, 5.6% in the kidney, 12% in the spleen, and 7.4% in the intestine. Only 10.2% of the injected dose had been excreted by urinary, biliary, and fecal routes after 24 hours of exposure. Distribution of  $^{64}Cu^{2+}$  was not affected by route of administration, with the exception of a higher concentration in the gills of fish exposed to the isotope in the water.

The object of this study was to assess the accumulation of Cu in the liver and kidney of rainbow trout (*Salmo gairdneri*) under field conditions. Trout were exposed to a concentration of CuSO<sub>4</sub> which would be effective for the control of algae, and used by fish farmers in Canadian prairie pothole lakes following guidelines from the Manitoba Department of Agriculture (1972).

#### METHODS

1,000 rainbow trout (Idaho strain) with a mean weight of 26.6 g, and 4,500 rainbow trout of the same strain with a mean weight of 6.9 g were obtained from the Rockwood Hatchery near Balmoral, Manitoba and transported approximately 250 km to Erickson, Manitoba. The two groups of fish were placed into nylon meshed cages in two pothole lakes (Lake 006 and Lake 108 respectively) to acclimatize for 10 days.

Partitions for these two potholes were made up of 6 ml polyethylene sheeting, which was joined together for proper length and depth by heat sealing. The sheeting was weighted at the bottom with lead line and anchors, and buoyed at the top with 1" x 2" wooden strapping. Anchors extended 90° from this strapping were utilized to keep wind and wave action from bowing or dragging the sheeting. Approximately half of the fish were released on each side of the partition in each pothole.

On June 14, 1976 one-half of Lake 006 was treated with 0.5 mg/L  $CuSO_{4.}5$  H<sub>2</sub>O (0.125 mg/L Cu), based on estimated volume of the partitioned half of the lake, and one week later Lake 108 was

treated in identical fashion. Water samples for cupric ion and total Cu analyses were taken from Lake 006 within 2 hrs of treatment, and again at 1, 2, 3, 4, 7 and 10 days. Lake 108 was sampled at 2 hrs, 1, 2, 3, 7, 9 and 22 days.

Cupric ion concentration of unfiltered lake water was analyzed with an Orion Ionalyzer (model 801) with an Orion cupric ion electrode (model 94-29) and an Orion reference electrode (model 90-04). Total Cu analyses of unfiltered lake water was performed using a Varian AA5 atomic absorption spectrophotometer with a model 63 carbon rod atomizer for concentrations <100  $\mu$ g/L Cu, and by flame atomic absorption spectrophotometry for concentrations >100  $\mu$ g/L Cu.

Fishing in L006 was accomplished with gill, trap, and hoop nets, whereas in L108 only trap and hoop nets were utilized. Fish samples were taken at the same time as the water samples. Immediately after capture, liver and kidney samples were removed and packed in ice. These samples were later digested using a modified sulfuric acidperoxide digestion procedure (Analytical Methods Committee 1967, 1976). Aliquots were analyzed for total Cu by flame atomic absorption spectrophotometry at the resonance line of 324.8 nm using a Varian AA5 spectrophotometer equipped with a model B-6 simultaneous background corrector.

Statistical analyses for L108 liver and kidney data were carried out by transforming the data into natural log form and treating them with a two-way analysis of variance (Sokal and Rohlf 1969). Because of inconsistent fishing success in L006, sample sizes were sometimes unequal and small. These data were not subjected to a two-way analysis of variance. Data are expressed as  $X\pm95\%$  confidence limits, where n≥3.

A follow-up study was done at a later date to determine if fish weight was correlated to liver and kidney [Cu]. Thirty-one rainbow trout (Idaho strain) of varying size were obtained from the Balmoral hatchery, and livers and kidneys from these fish were analyzed for total Cu using the methods previously described. Liver and kidney samples from the smallest 9 fish ( $\bar{X}$  wt. = 5.1±0.4 g) were pooled into 4 samples each. The remainder of the fish were sampled on an individual basis. Data were subjected to a linear regression using fish weight vs. tissue [Cu].

#### **RESULTS AND DISCUSSION**

The two lakes utilized in this study are moderately saline, of high pH, and with sufficient OH<sup>-</sup> and HCO<sub>3</sub> content (Table 1) to bind most of the added Cu into a complexed or insoluble form (Sillen & Martel 1964; Sylva 1976). Ionic Cu concentrations were proportional to total Cu, and represented about 30-50% of total Cu. Initial Cu concentrations were about two times higher than was expected from estimation of the lake volumes, and this was probably caused by a lack of uniform mixing due to surface application of CuSO<sub>4</sub>. [Cu] in the water decreased to about 50% within 2-3 days after application and to background levels within 10 days (Figs. 1 and 2). It is likely that uptake of Cu by algae, which were in the beginning stages of Cyanophyte bloom at the time of Cu addition, and subsequent sedimentation of the dead cells killed by the algicidal effect of Cu (McIntosh 1975; Whitaker et al. 1977), were important factors in the removal of Cu from the water column.

There was a small and unexpected increase in both total and ionic Cu on the 7th day after  $CuSO_4$  application in LOO6 (Fig. 2), which may have been caused by a partial remobilization of Cu from the sediment by wind action.

There were no significant changes in [Cu] in either the livers or kidneys of fish exposed to CuSO<sub>4</sub> (initial lake concentration of 125  $\mu$ g/L Cu) during the 10 day experimental period (Figs. 3 and 4). The LOO6 fish with a mean weight approximately 2.5 times that of the L108 fish, contained approximately 2.5 times more liver Cu than the L108 fish (Table 2).

A subsequent laboratory experiment (Fig. 5) showed a significant correlation (p<0.01) between fish weight and liver [Cu] in fish taken from the same hatchery and of the same strain as those used in this field study. No correlation existed between fish weight and kidney [Cu]. Cu is a component of the commerical trout food ( $\approx$ 11.0 µg/g Cu - Allard pers. comm.) fed to these fish while at the hatchery. Life (1970) claims that goldfish do not have the capacity to excrete Cu because they have relatively low levels of ceruloplasmin, a protein necessary for the excretion of this metal. If this is true for trout, it is likely that the larger individuals will have accumulated more Cu through their diet than the smaller ones.

From this short term exposure study it can be seen that rainbow trout do not accumulate Cu as a result of algal treatment. This is likely due to the fact that Cu is present at high concentrations only for a short time, as a result of the complexing action of substances within the water column. Since these lakes are land-locked (ie. effectively no drainage), repeated CuSO4 treatment may eventually cause a rise in lake [Cu] (Nicholset al. 1946). If high [Cu] were maintained in the water for longer periods of time, Cu accumulation in these fish could occur via the water (Brungs et al. 1973). However, considering the hardness and alkalinity of these lakes (Table 1), it is unlikely that a prolonged rise in water [Cu] would occur. McIntosh (1975) demonstrated that Cu added to an aquatic system is rapidly absorbed by the sediments, as well as by aquatic plants and algae, presenting the possibility of trout accumulating Cu through a food chain. He found a slight, temporary increase in green sunfish (Lepomis cyanellus) whole body [Cu] within 7-10 days of treatment with 3.0 ppm  $CuSO_4.5 H_2O$ , but the initial lake [Cu] was six times higher than the concentration used in our study, and 20-30 times higher than the recommended effective concentration (25-40  $\mu$ g/L Cu, Whitaker et al. 1977) for algal control.

In a long term exposure Lett et al. (1976) have demonstrated that rainbow trout exposed to 75-225  $\mu$ g/L Cu in hard water had a depressed appetite and growth rate from which it took about 40 days to recover. Since the rainbow trout stocked in the Erickson area have a residence time of  $\approx 5$  mo. (Lawler et al. 1974), the term of exposure is an important factor to consider when choosing the proper [Cu] for algal control. Choosing the relatively low concentration ( $25-40 \mu g/L Cu$ ) recommended by Whitaker et al. (1977) for algal control in these lakes, would result in minimal, if any adverse effects on rainbow trout over the 5 month residence time.

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Table 1. Chemical and morphometric characteristics of experimental lakes, nos. 108 and 006, Erickson, Southwest Manitoba (June, 1976).

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Lake	Specific Conductance µmhos/cm	рН	Na (mg/L)	K (mg/L)	Mg (mg/L)	Ca (mg/L)	C1 (mg/L)	SO4 (mg/L)	HCO3 (mg/L)	CO3 (mg/L)	Area (ha.)	₹ Depth (m.)	Max. Depth (m.)
108	1450	8.7	18	29	138	92	13	666	335	18	9.0	0.6	1.6
006	980	8.1	7.1	28	63	108	17	277	321	14	1.6	2.5	3.1

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Table 2. Mean [Cu] (ug/g wet weight) for rainbow trout sampled from lakes 108 and 006 over the 10 day experimental period. Data are expressed as  $\overline{X} \pm S.D$ .

Lake #	Kid	ney	Liver	:	·· · · ·	Length (cm)
	Control	Treated	Control	Treated	(g)	
108	1.75±.58 n=48	1.73±.29 n=48	20.93±8.05 n=42	20.62±10.90 n=42	15.1±6.6	10.2±1.3
006	1.42±.56 n=26	1.60±.59 n=27	51.33±20.51 n=27	54.85±14.60 n=27	37.6±11.3	14.2±1.5

Fig. 1. Lake 108. Concentration vs. time curves for total and ionic copper. Each point represents one sample taken at a 1.0 m depth.





Fig. 2. Lake 006. Concentration vs. time curves for total and ionic copper. Each point represents one sample taken at a 1.0 m depth.

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Fig. 3. [Cu] vs. time curves for livers from rainbow trout (Salmo gairdneri) sampled in Lake 006 and Lake 108. [Cu] is expressed as μg/g Cu wet weight. n=6 for all points in the L108 graph, and n is noted in individually over all points in the Lake 006 graph. Vertical lines represent 95% confidence limits.

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Fig. 4. [Cu]vs. time curves for kidneys from rainbow trout (*Salmo gairdneri*) sampled in Lake 006 and Lake 108. [Cu] is expressed as  $\mu g/g$  Cu wet weight. n=6 for all points in the L108 graph, and n is noted individually over all points in the Lake 006 graph. Vertical lines represent 95% confidence limits.



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Fig. 5. Liver [Cu] vs. fish weight plot for 31 rainbow trout (Salmo gairdneri) obtained from the Rockwood Hatchery, Balmoral, Manitoba. [Cu] is expressed as  $\mu g/g$  Cu wet weight.

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