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Acute and Sublethal Toxicity of Thallium to Aquatic Organisms

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A toxicity assessment was conducted to examine the effect of Cominco Trail outfalls on aquatic organisms in 1996. As a result of this study, thallium was implicated as a possible source of metal toxicity. Since limited scientific data is available regarding aquatic toxicity of thallium to vertebrates and invertebrates, Cominco contracted BC Research Inc. to evaluate the acute and sublethal toxicity of thallium to several freshwater aquatic organisms. Threshold values for thallium were derived for the first time for the test species used in this investigation.

The major sources of thallium (Tl) releases to water include nonferrous metals, iron and steel manufacturers and various mining, inorganic chemicals, refining, and ore-processing industries (EPA 1980, 1983; Ewers 1988). Thallium may also be present in the vicinity of smelters, particularly when thallium is not recovered in that process.

Since thallium is a naturally-occurring element, it may be present in ambient waters in trace amounts. However, monitoring data indicate that thallium concentrations are elevated near industrial and commercial sources and hazardous waste sites. The concentrations of thallium in river water that receives mining operation effluents ranged from 0.7 to 88.3 µg/L at locations in the United States and Canada (EPA 1980, 1988; Zitko and Carson 1975).

Thallium is a nonvolatile heavy metal that exists in water primarily as a monovalent ion (Tl^+); thallium may be trivalent (Tl^{3+}) in very oxidizing water (Callahan *et al.* 1979). Tl^+ forms complexes in solution with halogens, oxygen, and sulfur (Lee 1971). Thallium may precipitate from water as solid mineral phases. Wallwork-Barber *et al.* (1985) examined the transport of thallium among four basic aquatic components: water, sand, vegetation, and fish. It was found that thallium concentrations decreased slowly in the water and increased tenfold in the vegetation and fish. Definite transport of thallium occurred among water, fish, and vegetation, but no significant transport was seen between the sand and the other ecosystem components.

Thallium has been shown to bioconcentrate in the tissues of aquatic organisms from water. Bioconcentration factor (BCF) values of 18.2 for clams and 11.7 for

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mussels have been reported (Zitko and Carson 1975). Bioconcentration factors for the muscle tissue of juvenile Atlantic salmon are reported from 27 to 1,430 (Zitko and Carson 1975). The maximum BCF for bluegill sunfish was 34 in the study of Barrows *et al.* (1978). Thallium is absorbed by plants from soil and thereby enters the terrestrial food chain (Ewers 1988). Cataldo and Wildung (1983) demonstrated that thallium could be absorbed by the roots of higher plants from the rhizophere.

It was reported by Zitko and Carson (1975) that thallium was present in the effluents of the base-mining industry, and its acute toxicity to juvenile Atlantic salmon is approximately equal to that of copper and zinc and cadmium.

Little is known about the toxicity of thallium to aquatic animals. Thallium is known to kill fish slowly at concentrations of 1-60 ppm (Zitko and Carson 1975). It is lethal to fish in 72 hr at concentrations of 10-60 mg Tl/L (lethal concentration for rainbow trout is 10-15 mg Tl/L, LD 50 for Atlantic salmon is 0.03 mg Tl/L), and to aquatic insects and invertebrates at 2-4 mg Tl/L (Zitko and Carson 1975). The lethal concentration for tadpoles is 0.4 mg Tl/L (Zitko and Carson 1975). Algae may be affected at concentrations as low as 0.1 ppm (EPA 1980).

MATERIALS AND METHODS

The acute toxicity of thallium to fish and invertebrates is not well documented to date, although some phytotoxic effects have been observed (Overnell 1975). To determine the acute toxicity behavior of thallium to rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna,* laboratory bioassays were conducted to derive the LC50 values of thallium for these two species.

Sublethal effects of thallium were evaluated by survival and reproduction tests using *Ceriodaphnia dubia.* Test endpoints were survival concentration LC50 and reproduction inhibition concentration IC25. Since a phytotoxic response was expected for thallium, an aquatic algae growth inhibition (IC25) test was included.

All bioassays included negative controls and positive controls in the form of applicable reference toxicant tests run concurrently with tests. All analyses were conducted with the appropriate QA/QC measures used throughout the toxicity tests. Water samples were analyzed by ASL Ltd. in Vancouver.

Range finding tests for all species were conducted prior to the formal tests. Water samples were taken at the beginning and end of the tests for thallium analyses. In addition, a water sample was taken midway during the 7 day Ceriodaphnia test. Temperature, pH, and dissolved oxygen were also measured in the rainbow trout and *Daphnia magna* bioassays on the first and last days of the test.

The source of the rainbow trout, the organism holding conditions, the acclimation, and the test procedures were strictly based on the Environment Canada protocol (Environment Canada 1990a).

The nominal concentrations for the formal fish tests were 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 mg Tl/L. The 96 hour rainbow trout survival test was repeated five times following identical protocol. The rainbow trout used in the toxicity tests were weighed and measured following the conclusion of the tests (0.36 ± 0.08 g wet body weight; 3.4 ± 0.3 cm fork length).

Daphnia magna have been used extensively for evaluating the acute lethality of test materials under defined laboratory conditions. Standard or reference methods have been developed for determining the acute lethal toxicity of chemicals or effluents to the cladoceran "waterflea", *Daphnia magna*. The procedure used followed the Environment Canada methodology documents (Environment Canada 1990b).

The nominal concentrations for the formal tests were 0, 0.56, 1, 1.8, 3.2, 5.6, and 10 mg Tl/L. The 48 hour *Daphnia magna* survival test was repeated five times following identical protocol.

Ceriodaphnia dubia was used to conduct the 7-day survival test. In this test, *Ceriodaphnia* were exposed in a static renewal system until 60% of the control organisms have had three broods of offspring, usually 7 ± 1 days. The test endpoints were survival and reproduction. The Environment Canada methodology document (Environment Canada 1992b) was followed for the organism culturing, test performance and reporting.

The nominal concentrations for the formal tests were 0, 0.032, 0.063, 0.125, 0.25, 0.5, 1.0 mg Tl/L. The 7-day *Ceriodaphnia dubia* survival and reproduction bioassay was repeated three times following identical protocol.

The algal growth inhibition test using the microplate technique is a screening test for phytotoxicity that is used to increase the efficiency in the processing of samples, as compared to the classic algal bottle test. This study used the microplate technique and the freshwater alga *Selenastrum capricornutum* for an indication of plant toxicity of thallium.

The procedure followed for *Selenastrum capricornutum* tests provides explicit descriptions of a standard method for determining dose-dependent effects including the inhibition of algal growth over 72 hours. It gives specific instructions for performing and reporting algal growth inhibition tests and builds on the guidance provided in the generic methodology document (Environment Canada 1992a).

The nominal concentrations for the formal tests were 0, 0.032, 0.063, 0.125, 0.25, 0.5, 1.0 mg Tl/L. The 72-hr *Selenastrum capricornutum* bioassay was repeated five times following identical protocol.

In the multi-concentration tests for rainbow trout and *Daphnia magna*, the percentages of fish and daphnids killed at each concentration of thallium within

96 hr and 48 hr, respectively, were recorded. The 96 hr and 48 hr LC50s and their 95% confidence limits were calculated, respectively, for fish and daphnids. The Bioassay Reporting System Version 3.0 (1993-1994), developed by Bayleaf Software Inc., was used to perform these calculations and either probit or binomial methods were adopted as appropriate.

For the *Ceriodaphnia* (reproduction) and *Selenastrum* (growth) sublethal tests, an interpolated point estimate called the percentage inhibiting concentration (IC*p*) was used as the endpoint. In this study the IC25 and its 95% confidence limits was estimated by a linear interpolation method for sublethal toxicity, using a computer software (ICPIN version 2.0).

Dose response curves were prepared by pooling the test replicates for each organism using SigmaPlot® for Windows version 4.01 (1986-1997, SPSS Inc.). Response was plotted as probability or on a linear axis depending on the nature of the available data. Thallium concentrations were plotted on a log scale.

RESULTS AND DISCUSSION

The main test results are summarized in Table 1 and Figures 1-5. Chemical analyses of the high, middle, and low concentrations showed that actual Tl concentrations were at least 89% of target (nominal) concentrations. The nominal concentrations were used to generate the lethal and sublethal endpoints because they were not significantly different to measured concentrations.

The average 96 hour LC50 estimate for rainbow trout was 4.27 ± 1.40 mg Tl/L (Table 1). An LC50 of 3.2 mg Tl/L is indicated from a plot of the data from the five replicate experiments (Fig. 1). This result is similar to the estimated LC50 obtained from replicate 2 (Table 1) and within the confidence limits of other estimates. In the present study, 100% mortality was observed after 24 hours in 100 mg Tl/L concentration and death was seen in concentration as low as 25 mg Tl/L within one day of exposure. At 48 hours, 100% mortality was recorded in 25 mg Tl/L, and fish mortality was observed at the lowest concentration employed in the test (1.56 mg Tl/L).

Zitko and Carson (1975) reported an LC50 of 10-15 mg Tl/L for rainbow trout of similar size and life stage. However it appears that the juvenile rainbow trout used in the tests described in the present paper were more sensitive to thallium. This may be due to differences in (1) the specific test protocol, (2) test water properties such as hardness, or (3) organism sensitivity variation. The LC50 derived in this paper should to be viewed as a value generated based on to the test requirements from the Environment Canada fish bioassay protocols.

The average 48 hour LC50 for *Daphnia magna* was estimated at 2.01 ± 0.02 mg Tl/L (Table 1). A LC50 of 2.2 mg Tl/L can be estimated from the data for the three replicate tests plotted in Figure 2. A 7 day LC50 of 0.37 ± 0.01 mg Tl/L was

estimated from the *Ceriodaphnia dubia* tests, (Table 1; Fig. 3) which was not unexpected since this species is commonly found to be more sensitive than *D. magna*. The sublethal reproduction test results indicated that 0.10 ± 0.03 mg Tl/L thallium would cause the reproduction of *Ceriodaphnia dubia* to be reduced by 25 percent after 7 days of exposure (Table 1). Figure 4 shows this inhibitory effect of thallium on *Ceriodaphnia* reproduction.

Previous studies have shown that thallium can cause phytotoxic effects. Therefore, it is reasonable to expect that algae would be adversely affected at concentrations as low as 0.1 ppm (U.S. EPA 1980). Our results showed that for a freshwater algae species, *Selenastrum capricornutum*, the average value for inhibition growth for 25% of the population, IC25 was 0.09 ± 0.02 mg Tl/L (Table 1).

Table 1. Summary of bioassay results for thallium toxicity

Organism	Rep.	End Point	Result (mg Tl/L) (95% confidence)	Average±SD
Rainbow Trout	1	96-hr LC50	5.50 (1.11, 20.97)	
(mortality)	2	96-hr LC50	3.27 (1.77, 4.98)	
	3	96-hr LC50	2.77	
	4	96-hr LC50	3.84 (0.0, 18.57)	
	5	96-hr LC50	5.97 (1.45, 23.57)	4.27 ± 1.40
Daphnia magna	1	48-hr LC50	1.94 (1.0, 3.2)	
(mortality)	2	48-hr LC50	1.94 (1.0, 3.2)	
	3	48-hr LC50	1.75 (1.3, 2.3)	
	4	48-hr LC50	2.16 (1.0, 3.2)	
	5	48-hr LC50	2.26 (1.8, 3.2)	2.01 ± 0.02
Ceriodaphnia dubia	1	7-day LC50	0.3 (0.3, 0.5)	
(mortality)	2	7-day LC50	0.4 (0.3, 0.5)	
	3	7-day LC50	0.4 (0.1, 0.5)	0.37 ± 0.01
Ceriodaphnia dubia	1	7-day IC25	0.14 (0.03, 0.16)	
(reproduction)	2	7-day IC25	0.06 (0.03, 0.16)	
	3	7-day IC25	0.09 (0.08, 0.13)	0.10 ± 0.03
Selenastrum	1	72-hr IC25	0.06 (0.01, 0.08)	
capricornutum	2	72-hr IC25	0.12 (0.01, 0.21)	
(growth inhibition)	3	72-hr IC25	0.10 (0.01, 0.14)	
	4	72-hr IC25	0.10 (0.07, 0.15)	
	5	72-hr IC25	0.08 (0.01, 0.19)	0.09 ± 0.02

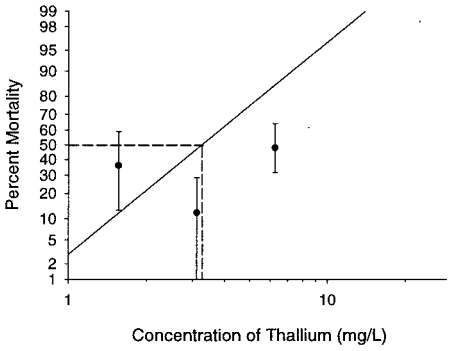

Figure 1. Effect of thallium on rainbow trout mortality

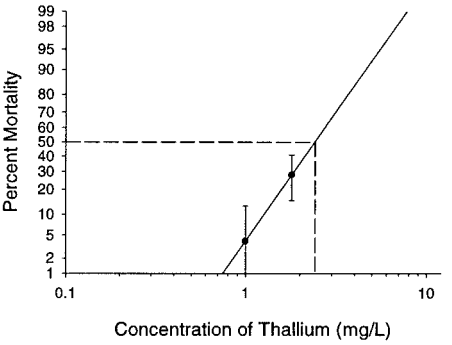

Figure 2. Effect of thallium on *Daphnia magna* mortality

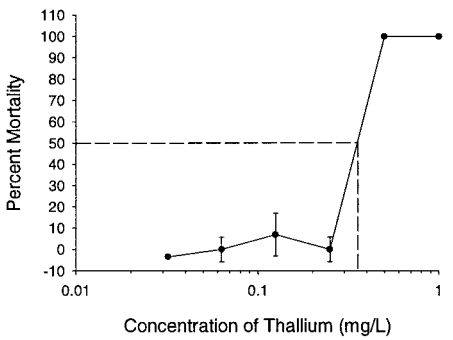

Figure 3. Effect of thallium on *Ceriodaphnia dubia* mortality

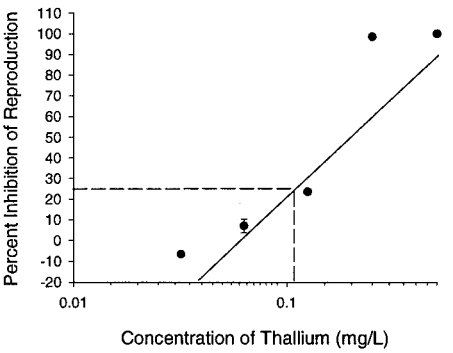

Figure 4. Inhibitory effect of thallium on *Ceriodaphnia dubia* reproduction

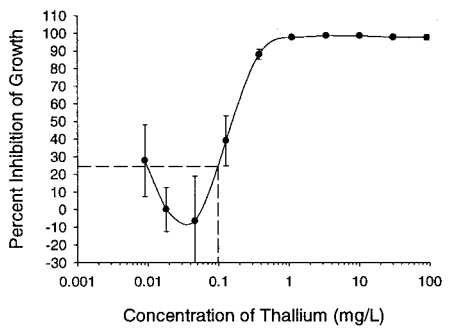

Figure 5. Inhibitory effect of thallium on *Selenastrum capricornutum* growth

Figure 5 shows the inhibitory effect of increasing thallium concentrations to *Selenastrum* growth. An IC25 of 0.1 mgTl/L can be estimated from this concentration response curve. An interesting response pattern can be observed which suggests that thallium may stimulate algal growth at concentrations ranging from 0.01 to 0.03 mg Tl/L (Fig. 5). However, there is considerable variability in this response data as indicated by the error bars. Therefore, further experiments would be required to confirm this stimulatory effect.

The concentration which caused an inhibition of growth in 25% of the population (IC25) was chosen as a primary endpoint for the sublethal tests. This statistic provides an estimation of a single concentration which causes a specified effect, rather than the pair of concentrations represented by NOEC (No-Observed-Effect-Concentration) and LOEC (Lowest-Observed-Effect-Concentration). A major disadvantage of the NOEC/LOEC is that no variance can be calculated, and so no confidence limits can be given for the estimates. In addition, the NOEC/LOEC must be extrapolated from the data, and do not provide any information regarding the slope of the dose response curve. In most cases, IC20 or IC25 would be closer to the NOEC, however, there is more statistical precision surrounding the IC50 and thus greater confidence in the point estimate.

The concentrations which caused measurable sublethal effects (IC25) to *Ceriodaphnia dubia* reproduction and *Selenastrum capricornutum* growth were remarkably similar, approximately 0.1 mg/L. Therefore, this concentration level could be used as a reference point to develop water quality criteria for the protection of freshwater aquatic species. This concentration of thallium would also be protective for preventing acute mortality to rainbow trout and *Daphnia magna*.

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