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# Oxygen Consumption of Juvenile Rainbow Trout (Oncorhynchus mykiss) Exposed to Sublethal Concentrations of 1,2,4,5-Tetrachlorobenzene and Tetrachloroguaiacol

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The rate of transfer of hydrophobic toxicants across fish gills increases with increased oxygen consumption (Brauner *et al.* 1994; Yang and Randall 1995). Fish oxygen uptake, which is easy to measure and is well documented in the literature, may be utilized in modeling toxicant uptake. Although oxygen uptake of fish has been suggested as an index of sublethal toxicity (Sprague 1971), there have been a number of studies showing that there was little influence on oxygen uptake of rainbow trout exposed to 1,2,4,5-tetrachlorobenzene (TeCB) (Brauner *et al.* 1994), or of coho salmon exposed to some herbicides (Johansen and Geen 1990; Janz *et al.* 1991) and wood preservatives (MacKinnon and Farrell 1992). Except for some studies on their acute toxicity (USEPA 1990), physiological impacts on osmoregulation (Yang and Randall 1996), swimming performance (Brauner *et al.* 1994), and bioaccumulation (Tischmak 1984; Brauner *et al.* 1994), there is little information on the sublethal effects of TeCB and a related compound, tetrachloroguaiacol (TeCG), on fish. The objective of our study was to look at the effect of prolonged sublethal exposure of TeCB and TeCG on the oxygen uptake of juvenile rainbow trout *(Oncorhynchus mykiss)* using a newly modified, multi-vessel, computerized flow-through fish respirometer (Duval *et al.* 1981; Johansen and Geen 1990).

## MATERIALS AND METHODS

Rainbow trout (43.8 ± 3.6 g; fork length 15.2 ± 0.3 cm), were purchased from West Creek Trout Farm, Aldergrove, British Columbia. Fish were held in a 2.5 meter diameter fiberglass tank under natural photoperiod in dechlorinated Vancouver city water (temperature 5-10 °C, pH 6.1-6.3, hardness 5.2 to 6.0 mg/L as $CaCO_3$ and $O_2$ saturation approx. 95%) at Simon Fraser University, Burnaby, British Columbia. They were fed commercial trout pellets, Biodiet Grover (purchased from Bioproducts, Warrenton, Oregon), weekly ad libitum. They were starved at least 96 hr prior to the trials to assure a post-absorptive state (Goss and Wood 1988; Beamish 1978) and were allowed a 24 h acclimation period within the glass vessel before the initiation of each test.

TeCB and TeCG are chlorinated compounds formed in the bleaching processes of the pulp and paper industry (Garrett 1980). Both TeCB and TeCG are relatively hydrophobic with the log $K_{ow}$ being 4.97 and 4.41, respectively (USEPA 1990). Although their water solubility was sufficient to make them bioavailable in the water, acetone was used to facilitate their dissolution in the test medium. Acetone, used as a chemical carrier, has been proved to be non-toxic to fish at the concentration (1 : 3806 v/v) administered in our study (Johnson and Finley 1980). TeCB and TeCG were acquired from Pfalz and Bauer

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Inc., Stamford, Connecticut, and Chem Services, West Chester, Pennsylvania, respectively.

All tests were conducted in a six-glass-vessel computer-controlled, intermittent-flow fish respirometer (Fig. 1) originally designed by Duval *et al.* (1981) and modified subsequently by Johansen and Geen (1990). The volume of each glass vessel was around 8 L and was covered with opaque polyethylene during the whole test period to minimize possible disturbance. The oxygen readings were given by the OxyGuard® dissolved oxygen (DO) probe, mounted through the lid into each glass vessel, instead of the modified YSI Model 53 oxygen monitors used by Johansen and Geen (1990). A transduction 16 bit, 64K DEC LSI 11/02 compatible computer and a custom made A/D-D/A interface were used for system control and data acquisition. Preliminary trials were conducted to find out the optimal total fish body weight each vessel could accommodate in order to avoid any possible continuous oxygen deficit or interactive stress within each vessel, and to assure DO measurement accuracy. Four fish (~ 200 g in total body weight) were placed in each vessel and acclimatized for 24 hr under continuous flow conditions before each experiment. All vessels were immersed in a 175 L water bath to minimize water temperature fluctuations (7.11 ± 0.14 °C). The fluorescent lights produced a simulated 14 : 10 hr light : dark photo-period. Parameters such as pre-exposure time, exposure time, cycle time, measurement frequency, purge time/rate, bypass time, toxin flow rate, toxin stock solution concentration, and toxin pump time, etc., were manipulated in order to achieve a predetermined toxicant exposure regime.

Two tests, one with TeCB and one with TeCG, were carried out under identical conditions. Each trial lasted for 96 hr, with both the pre-exposure and exposure period being 48 hr. Each vessel was flushed with freshwater every 15 min with the purge rate at 1.0 L/min for 2.0 min. The oxygen probe inserted into each vessel was set to record the oxygen level in each vessel every 5 min. The bypass time between each purge was 10 sec, when the connecting tubes were flushed to prevent any possible toxicant residue accumulation. The toxicant flow rate was 2 mL/min and the concentration of the toxicant stock solution was 100 mg/L TeCB or TeCG. The toxicant was pumped into each vessel, where required, during a purge. The pump time was adjusted, respectively, at 0 min for two control group vessels, 1.0 min for two 100 µg/L treatment groups and 2.0 min for 200 µg/L treatment groups. Eight control fish at the beginning and all fish in the vessels at the end of each test were killed by a sharp blow to the head. Body weight and fork length were then measured.

Completion of the sequential oxygen measurements and freshwater flushing in six vessels constituted one cycle. Oxygen consumption rate was based on the pooled value for four fish in each vessel, and total fish body weight in that vessel was measured at the end of the 48 hr exposure when the fish were killed. The average of oxygen consumption in every hour was calculated and the oxygen consumption data profile analysed between control and treatment groups, or within treatment groups but between 48 hr pre-exposure and 48 hr exposure periods. One way ANOVA followed by a Dunnett's test or student *t*-test were used to determine if there was any effect of the two chemicals on the oxygen consumption pattern of the fish.

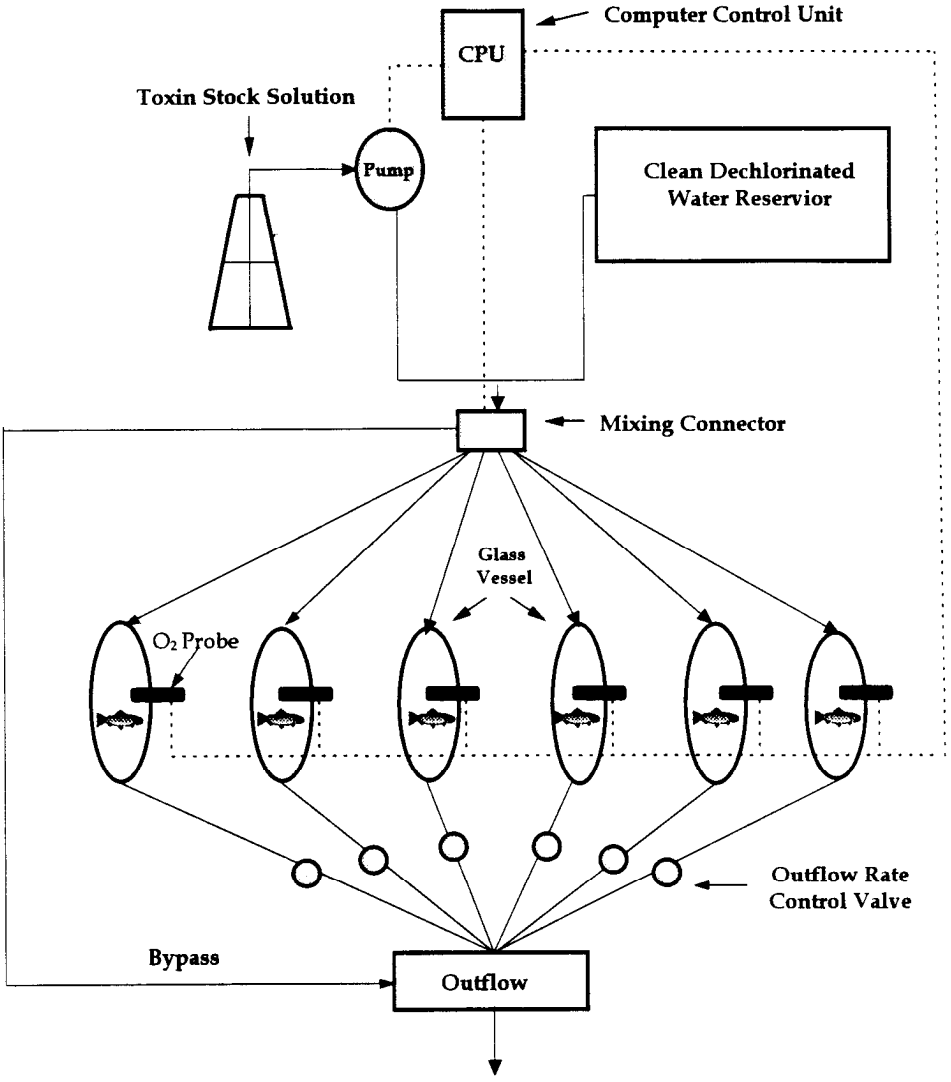


Figure 1. A schematic diagram of the six-vessel, intermittent flow-through, computerized fish respirometer

## RESULTS AND DISCUSSION

A major modification in this flow-through respirometer compared with that described by Johansen and Geen (1990) was the use of the OxyGuard® DO probe, which has a built-in temperature compensation, a large anode and electrolyte volume. Probe air calibration was performed according to the manual (Point Four Systems Inc.) prior to each experiment. The probe used very little oxygen for its measurement and, therefore, enabled it to function correctly with liquid movement as low as 2 cm/set (measured at 7 ppm and 13 °C). It was demonstrated in the pre-experimental trial that the total amount of oxygen the probe consumed by itself was minimal (Table 1) during 20 min which was around the time duration of each cycle.

Table 1. Oxygen consumption by the OxyGuard® probe during 20 min at 7.07 °C.

| | *Probe 1* | *Probe 2* | *Probe 3* | *Probe 4* |
|---|---|---|---|---|
| Dissolved $O_2$ (ppm) | 8.09 | 8.51 | 10.29 | 10.41 |
| | 7.88 | 8.33 | 10.24 | 10.35 |
| | 7.77 | 8.20 | 10.21 | 10.31 |
| | 7.72 | 8.13 | 10.19 | 10.29 |
| | 7.70 | 8.10 | 10.18 | 10.28 |
| % Decrease in 20 min | **4.8** | **4.8** | **1.1** | **1.2** |

Marked respiratory changes occur in fish under hypoxic conditions (Hughes 1973; Randall 1982; Bushnell *et al.* 1984). The number of fish each vessel could accommodate was another parameter that could have potentially affected the survival of the test fish or the accuracy of oxygen uptake measurements. In pre-experimental trials, four or six fish were put into a glass vessel and oxygen depletion was measured after 20 min. A 20.7% drop of dissolved oxygen was detected in the vessel of four fish and 35.7% in the vessel of six fish. After 5 min freshwater flushing the DO level in the vessel containing four fish went back to 102.8% of the original level, while the DO was only 84.1% of the initial concentration when six fish were present in the chamber. Thus, only four fish were included in each 8 L vessel during the actual experiments.

A characteristic circadian rhythm in fish oxygen consumption rate was detected in control fish (Fig. 2a & 2b), reflecting the normal daily activity pattern (Brett and Zala 1975) of the fish used in our study. The mean fish oxygen consumption value was similar to values reported by other investigations using similar apparatus (MacKinnon and Farrell 1992; Janz *et al.* 1991; Johansen and Geen 1990) indicating that the system could provide reliable oxygen recordings with the newly installed OxyGuard® DO probes. The DO detecting system was the major modification of the old respirometer whose oxygen consumption data was generated using the averaged readings of four inflow and outflow electrodes (Johansen and Geen 1990). By directly monitoring the oxygen concentration in the vessels, the calculation involved for oxygen consumption was simplified and, more importantly, the accuracy of the measurement was improved.

After being acclimated in the vessel for 48 hr, rainbow trout exposed to 100, 200 µg/L TeCB (Fig. 2a) or TeCG (Fig. 2b) did not show any statistically significant differences in oxygen consumption throughout the 48 hr exposure period. This conclusion was based on

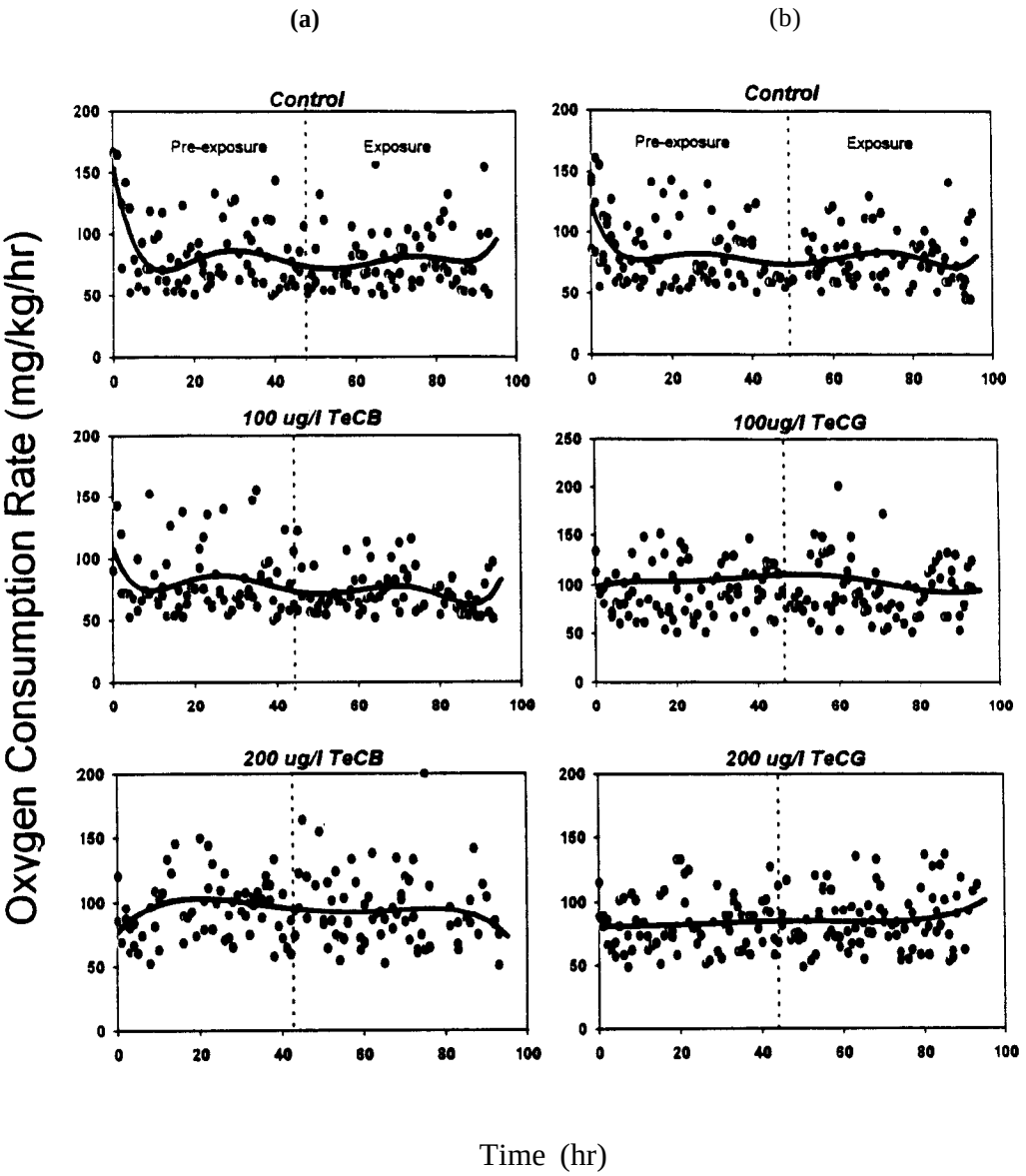


Figure 2. Effects of TeCB and TeCG flow-through exposure on the oxygen consumption of juvenile rainbow trout (*Oncorhynchus mykiss*). Each point represents the mean value of fish oxygen uptake rate at 30 min intervals.

the comparison between the fish oxygen uptake profile before and after exposure in the same group ($p<0.05$), and those in the control and treatment groups ($p<0.05$). Similarly, MacKinnon and Farrell (1992) have shown that a concentration-dependent response in oxygen consumption of juvenile coho salmon (*Oncorhynchus kisutch*) was not observed with sublethal exposure to 2-(thiocyanomethylthio) benzothiazole. Thus, the results of our study and that of MacKinnon and Farrell (1992) indicate that oxygen consumption may not be a good indicator of sublethal chemical contaminant stress in fish.

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