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Growth inhibition in Japanese medaka (*Oryzias latipes*) fish exposed to tetrachloroethylene

Hattie B. Spencer[1, 2], Wedad R. Hussein[3] and Paul B. Tchounwou[1]
[1]Environmental Toxicology Research Laboratory, NIH-Center for Environmental Health, College of Science, Engineering and Technology, Jackson State University, Jackson, MS 39217, USA
[2]Department of Natural Sciences and Environmental Health, Mississippi Valley State University, Itta Bena, MS 38941, USA
[3]Department of Chemistry, School of Science and Technology, Jackson State University, Jackson, MS 39217, USA

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Abstract: *A recent study in our laboratory has demonstrated that tetrachloroethylene (TCE) is acutely toxic to Japanese medaka (Oryzias latipes) larvae with a 96 hr-LC_{50} of 18 (17-19) mg/mL (Spencer et al., 2002). In the present study we hypothesize that TCE exposure induces a developmental effect in Japanese medaka. Growth and age specific sensitivity of Japanese medaka larvae were studied with four age groups (7, 14, 21 and 28 days old) to determine tetrachloroethylene effects on these parameters. The medaka larvae were exposed for 96 hours in a single concentration (10 mg/mL) of TCE. The toxic endpoints evaluated were larvae weight, length, water content and protein concentration. The study revealed that exposure of medaka larvae to this sub-acute concentration of TCE significantly reduced length and weight in the treated group. The difference in growth between control and treated groups was more obvious in age versus length, than in age versus weight. The dry weight-fresh weight ratio (dw/fw) was shown to be higher in the control group. Water content in TCE-treated medaka was higher than in the control group, and younger fry had more water content than older ones. A higher protein concentration was also observed in TCE-treated medaka compared to the control group. These results indicate that TCE has a profound effect on the growth and development of Japanese medaka larvae.*

Key words: *Tetrachloroethylene, Toxicity, Japanese medaka, Growth inhibition.*

Introduction

Growth retardation has been shown to be one of the most detrimental and sublethal effects observed in ecotoxicological studies. Growth is an important factor in toxic responses because reduced growth may affect competition for food and habitat time to maturation, and susceptibility to predation and diseases (Woltering, 1984).

Studies have indicated that larval growth is a sensitive measure of toxic stress and that many fish subjected to sublethal levels of toxicants show a general decrease in total body length (Wilson 1976; Johnson *et al*., 1979; Norberg and Mount, 1985; and Norberg-King, 1989). Changes in growth produced by exogenous chemicals usually have multiple causes. Therefore growth is a sort of integrator of a variety of physiological and environmental factors (Weathering, 1990). Measurement of larval growth and macromolecular content after toxicant exposure have shown a high correlation with the early life stage growth which determines the fish size. It has been shown that protein content is reduced in a dose-dependent manner (Buckley, 1979; 1980). Toxicant effect on growth could be related to an inhibition of energy-yielding processes, channeling of energy for maintenance of homeostasis, or interference with appetitive behavior (Drummond *et al.,* 1973).

The Japanese medaka larvae have been used extensively in research due to short latency period for the induction of structural anomalies (Kirchen and West, 1976; Shi and Faustman 1989; Spencer *et al.,* 2002). In this study, static renewal bioassays were performed to assess the acute toxicity of tetrachloroethylene, and to evaluate it sub-acute effect on larval growth rate and protein content in the Japanese medaka, *Oryzias latipes*.

Weight and length have been used as a measure of growth in fish. Wilson (1976), Johnson *et al*. (1979), Norberg and Mount (1985), and Norberg-King (1989), indicated that fish growth may be a sensitive measure of toxic stress and that fish subjected to sublethal levels of toxicants show a general decrease in total body length. There are three major constituents that normally add weight to fish: lipid, water and protein contents. In this study a change in total protein concentration was used as a biochemical indicator to determine tetrachloroethylene effects on growth. This analysis examined whether changes in total protein content is a general response to changes in growth rate.

Materials and Methods

The test substance, tetrachloroethylene (99% purity) was of analytical grade. It was purchased from Sigma Chemical Company (St. Louis, MO.). Chemicals (sodium chloride, magnesium sulfate, potassium chloride, and calcium chloride) for preparing the embryo rearing medium were obtained from J.K. Baker Chemical Company (Phillipsburg, NJ). Protein reagent (Pierce Coomassie Plus Protein Assay Reagent Kit) was purchased from Pierce Company (Rockford, IL).

All experiments were conducted in an isotonic medium. The water temperature was maintained at 25ºC. A synthetic rearing medium (Rugh, 1962) was used to rear medaka

Table – 1: Mean values and standard deviations of body weights and lengths (by age group) of Japanese medaka fry pre-exposed to 10 ppm of tetrachloroethylene for 4 days. N =100.

Age	Body weight (mg)		Body length (mm)	
(Days)	Control	Treated	Control	Treated
7	0.005 (0.003)	0.003 (0.001)	6.5 (0.7)	6.0 (0.8)
14	0.008 (0.001)	0.004 (0.002)	7.0 (0.5)	7.0 (0.6)
21	0.010 (0.002)	0.006 (0.002)	10.0 (1.2)	9.0 (1.5)
28	0.018 (0.002)	0.009 (0.002)	12.0 (1.0)	9.0 (1.4)

embryos through hatching. All solutions were steam-sterilized in an autoclave (121ºC, 20 phi, and 15-20 minutes) or boiled for five minutes, cooled and stored at room temperature**.**

Water soluble fraction of tetrachloroethylene was prepared by adding the desired amount of tetrachloroethylene to 250 mL of rearing medium in a separatory funnel. The stock solution was mixed by shaking vigorously for 10 minutes. This procedure produced the maximum amount of tetrachloroethylene dissolved in a saturated solution without creating a tetrachloroethylene-water emulsion (Stoss and Haines, 1979). The solution was allowed to stand for 24 hrs allowing the tetrachloroethylene droplets to fall to the bottom of container. The stock was diluted with the rearing medium to produce the desire concentration of 10 ppm. The concentration of tetrachloroethylene in test solutions was determined by gas chromatographic method at the Department of Environmental Quality (DEQ) in Pearl, MS. Mortality was recorded every 24 hrs for the duration of the test.

Age specific toxicity was determined with four age groups of larvae (7, 14, 21 and 28 days old). Larvae were exposed for a time period of 96 hrs at a concentration of 10 ppm to determine tetrachloroethylene effects on growth rate and total protein in different age groups.

Weight and length of medaka larva at 7, 14, 21, and 28 days old were measured to determine the effects of tetrachloroethylene on larval growth. The wet weight and length of each fry were measured before and after 96 hrs of exposure and recorded at each measurement. The initial weight and length of all larvae in the study were determined from a subsample of 20 fish. Fry from both control and treatment groups were pipetted from the test chamber and assessed for total weight. Groups of fry (treated and control) were transferred to tared weighting trays and dried at 60°C for 24 hr in an oven (Serial No. 7860060 Sargent-Welch Scientific Co.). Samples were weighted to the nearest milligram on an analytical balance (Barron and Adelman, 1984). The fry were sacrificed in ice cold bath deionized water and stored in refrigerator for protein analysis.

Tissue homogenate of the Japanese medaka fish was used to determine total protein. Tissues obtained from tetrachloroethylene-treated and control groups were dry blotted twice to remove all water. Whole fish was weighted (average weight of .026g) and homogenized in 0.5 mL of 0.25M sucrose (C12 H22 O11) using a hand held tissue grinder (Kontes Glass Company, 885451-0023 and 885452-0023, Vineland, NJ) followed by centrifugation at 10,000 rpm for 10 minutes. The top layer of supernatant was removed and discarded. Fifty microliters (50µl) of the supernatant was pipetted into standard glass labeled test tubes.

The Pierce Coomassie Plus Protein Assay Reagent kit was used to determine total protein content in the medaka fish. The Coomassie reagent contained the Coomassie dye, methanol, phosphoric acid and solubilizing agents in water. The protein stock solution contained bovine serum albumin (BSA) at a concentration of 2.0 mg/mL in a solution of 0.9% saline and 0.05% azide solution.

Results and Discussion

Study was conducted to determine tetrachloroethylene effects on growth and age specific sensitivity of medaka larvae at ages 7, 14, 21, and 28 day-old. Larvae were exposed to a single dose concentration of 10 pm of tetrachloroethylene for 96 hrs. The mean lengths, weights and standard deviations for medaka larvae in the study are presented in Table 1. It has been reported that growth retardation is one of the most frequently observed ecotoxicological responses in freshwater pollution studies (Woltering, 1984). Embryonic metabolism has been reported to increase during exposure to toxicants thereby leaving little substrate material for growth (Anderson, 1974).

The difference in growth between control and TCE-treated groups was more obvious for age versus length than for age versus weight. Studies have revealed length to be a more sensitive indicator of growth than weight. The dry weight-fresh weight ratios (dw/fw) in both the control and treated are shown in Fig. 1. The increase in dw/fw was gradual in both groups but was significantly higher in the control than in treated groups.

As a fish grow, changes in weight have been shown to be greater than changes in length. In contrast to a change in length, a change in weight may be a transient indicator of

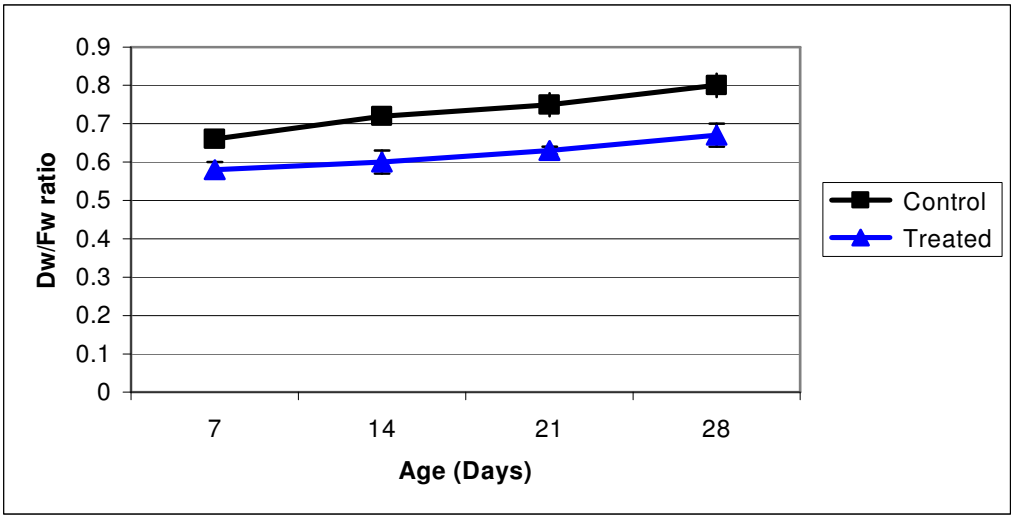

Fig. 1: Relationship between medaka larvae dry weight and fresh weight ratio versus age for the control and treated group exposed to 10 ppm tetrachloroethylene for 96 hours. Data points represent means ± SDs.

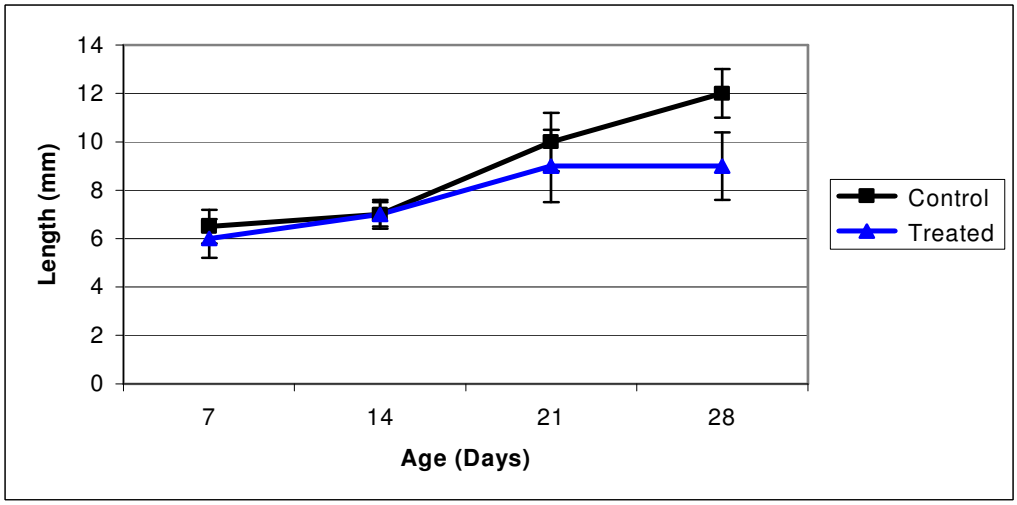

Fig. 2: Relationship between age and length in medaka larvae pre-exposed to tetrachloroethylene. Data points represent means ± SDs.

growth, therefore, weight may not be a suitable measure in growth studies (Busacker *et al.,* 1990).

A positive relationship was observed in lengths and weights of the control group (Fig. 2) within the age range studied. Growth rate was greatest between Days 14 and 28, which could represent a period of increase in metabolic rate. For the treated group, a decrease in the fry weights was observed at all ages. However, no length increase was noted between 21 and 28 day-old fry after reaching 9 mm, but the weight continue to increase (Fig. 3). This graph shows that fish in the control group grew faster than in the treated ones. Statistical analysis using the paired *t-test* showed a significant difference ($p < 0.05$) between the control and each of the treated groups. This difference in growth is attributed to the effect of the chemical, since it put a high stress on the treated fish resulting in energy being used to fight the stress instead of being used for growth.

The water content in the treated was higher than in the control (Fig. 4), and it was also noted that the younger fry had more water content than older ones. The increase in water content in the treated fry could be attributed to mild yolk-sac edema. A change in water content is not considered to be growth, however, if a fish gains water because its water balance mechanisms fail, weight will increase even though it has not added new body tissue. Weight can also increase due to change in body lipids, which may or may not be considered as growth (Busacker *et al.,* 1990).

Growth can be defined as the lying down of flesh through the process of protein synthesis (Heath, 1995). Based on this definition, total protein was extracted and quantified to

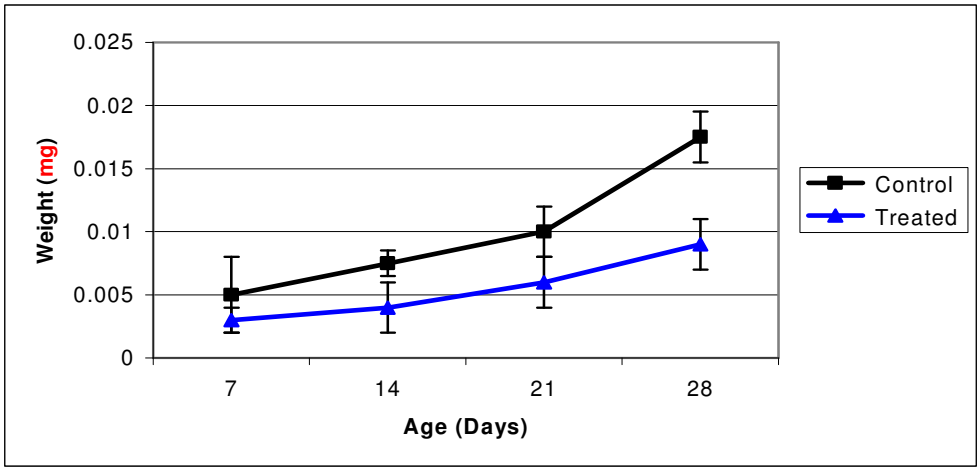

Fig. 3: The relationship between age and weight in medaka larvae pre-exposed to tetrachloroethylene. Data points represent means ± SDs.

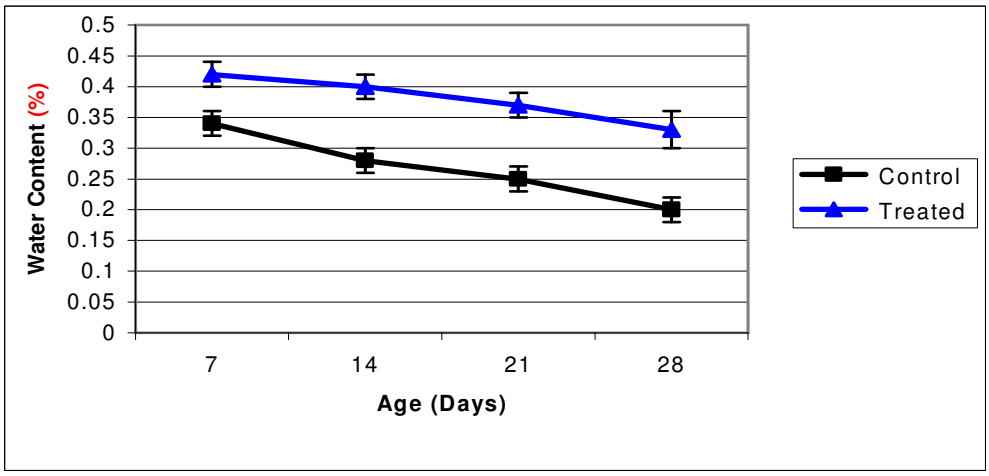

Fig. 4: Relationship between age and water content in medaka larvae pre-exposed to tetrachloroethylene. Data points represent means ± SDs.

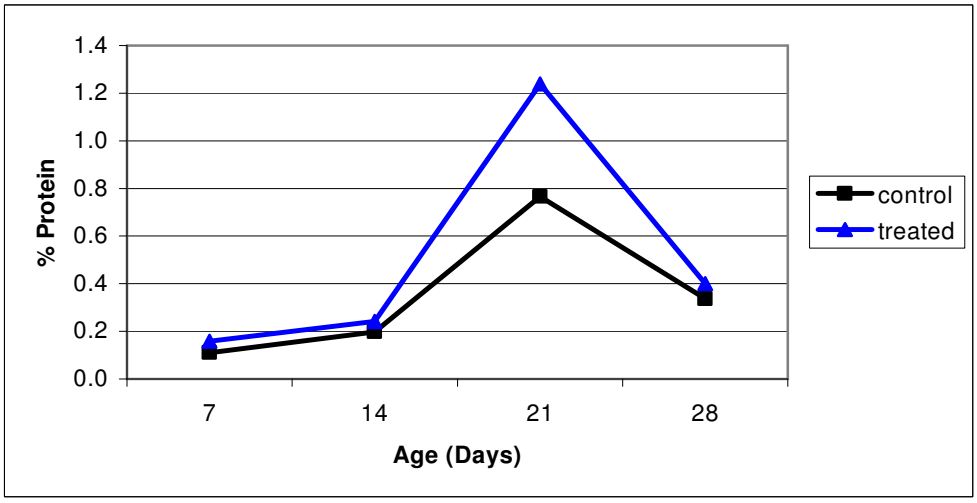

Fig. 5: Comparison of protein content in control versus treated medaka larvae pre-exposed to tetrachloroethylene.

see if the difference in weight between control and TCE-treated fish was due to the differences in protein content. The result shows that treated fish had higher protein content than that of the control group (Fig. 5). This is contrary to the results of the growth study, which shows a higher growth rate in the control than in the treated. Heath (1995) reported that unlike mammals, fish utilized protein for energy to a greater extent than do mammals but when they are under acute stress, they are somewhat similar to other vertebrate in that they mobilize and use carbohydrates. Since the treated fish were under chemical stress, they are more likely to use carbohydrates for their energy requirements while the control fish used most of their proteins. This difference could explain the high protein content in treated than in the control fish. Additional studies are needed to confirm this result and also to elucidate the underlying mechanism behind it (Heath, 1995).

In conclusion, this study revealed that a single sub-acute concentration of tetrachloroethylene (PCE) is toxic to the Japanese medaka fry, and has profound effect on growth, weight, length and protein content. Exposure to a sub-lethal concentration of 10 ppm PCE resulted in a significant reduction in weight and length, with control fish growing faster than treated fish. The increase in water content observed in treated fish is not an indication of weight gain, but more less attributed to mild yolk-sac edema. Protein content was shown to be higher in the treated groups. Although growth rate was faster in control fish, higher protein content was noted in treated fish.

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Correspondence to :
Dr. Paul B. Tchounwou
Presidential Distinguished Professor & Deputy Director,
NIH-Center for Environmental Health, College of Science,
Engineering and Technology, Jackson State University,
P.O. Box 18540, Jackson, MS 39217, USA
E-mail: paul.b.tchounwou@jsums.edu
Tel.: +1-601-979-3321
Fax: +1-601-979-2349