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Effect of Exposure to Several Pentachlorophenol Concentrations on Growth of Young-of-Year Largemouth Bass, *Micropterus salmoides*, with Comparisons to Other Indicators of Toxicity

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Pentachlorophenol (PCP) is a general metabolic poison used extensively as a biocide in many industrial applications and as such is a contaminant in many bodies of water (Rao 1978, Jones 1981). A primary action of PCP is the uncoupling of oxidative phosphorylation (Weinbach 1957, Weinbach and Garbus 1965) along with other effects on energy metabolism (Bostrom and Johansson 1972). The net effect is to increase the rate of energy metabolism in compensation for the reduced efficiency in ATP production. One important consequence of this is a reduced rate of growth , Kreuger et al. 1966, Webb and Brett 1973, Hodson and Blunt 1981, Holcombe et al. 1982, Sloof and Canton 1983, Mathers et al. 1985).

In a previous study (Mathers et al. 1985) we found that food conversion efficiency and growth of juvenile largemouth bass were reduced by a 14 day exposure to 50 ug/L PCP. We now wish to report on an investigation into the concentration-response relations of PCP over the short term (14 days), and compare the results to the effects of a longer term (52 days) exposure. From the data we determined the response threshold for a PCP effect on food conversion efficiency and compared these results with our other studies into the effects of PCP on behaviour and mortality.

From the present study it is clear that the PCP effect on food conversion efficiency has a rapid onset, under 14 days, and that the threshold level is below the US-EPA criterion level reported in Yount and Richter (1986).

MATERIALS AND METHODS

Yolk-sac fry were collected from male-defended nests and held in running water aquaria at the Queen's University Biology Station on Lake Opinicon, Chaffey's Locks, Ontario, Canada. Once the free-swimming stage was reached the fry were redistributed amongst several holding aquaria or placed under experimentation. All fry were aged from the date that 50% of the yolk-sac fry of an aquarium became free swimming.

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Fry in the holding aquaria were fed three times daily on live zooplankton from fertilized rearing pools and live brine shrimp nauplii. Once the fry were large enough, frozen brine shrimp was fed. All holding and experimental containers were cleared of accumulated debris two or three times daily. The 100 L holding aquaria were made of grey-painted wood with one glass side. All aquaria were supplied with lake water (alkalinity 65 mg/L as $CaCO_3$, (Smol, 1984); pH = 7.7) and a natural photoperiod. The water temperature fluctuated from 18°C up to 24°C.

To prepare the PCP stock solution 500 mg of reagent grade pentachlorophenol (lot no. 102F-004, Sigma Chemical Co., St. Louis, Mo., U.S.A; purity 99% by gas chromatography; contaminants not identified) was dissolved in 10.0 ml of 1.0 N NaOH solution and then diluted with distilled water to a total volume of 1.0 L. For the food conversion efficiency experiment the PCP exposure concentrations, expressed as mean µg/L (standard deviation), were 0, 1.6 (0.4), 13.0 (2.0), 48.7 (6.0), 72.4 (5.6) and 96.4 (3.9). These represent mean daily values over the course of the experiment calculated from the measured flow rates of the proportional diluter. The accuracy of the dosing method has been confirmed by gas-liquid chromatography of treated water in an earlier study. The analyzed values were the same as or up to 10% lower than the calculated values (Johansen et al. 1985).

Fry, 35 days old, were placed in the experimental containers and allowed seven days to acclimate, after which the seven day food conversion efficiency (as g of growth/g of food eaten) experiment was started. The fish were individually housed in 1.5 L plastic containers with nylon mesh sides and bottoms which allowed water circulation but confined the fish and their food. Ten containers were placed in the plastic 20 L treatment tanks. The fish were fed live adult brine shrimp. Uneaten shrimp, all still alive, were removed two hours after feeding. The weight of food eaten by each individual fish was calculated from the difference between the wet weight of the uneaten food and the wet weight of the food supplied. The fish were weighed live (to 1 mg) before and after the experimental period and the weight increase calculated. Fish were not fed 12 h before weighing. This protocol has been used before with larger bass feeding on live fish prey (Mathers et al. 1985). At the beginning of the experimental period the fish were not significantly different in size (overall mean weight = 342 mg, SD = 85. One way ANOVA $F = 0.13$, $P > 0.720$, d.f. 5, 54; overall mean total length = 36.9 mm, SD = 2.5). For within experiment comparisons, Duncan's multiple range test was used.

Fry, 52 days of age, from a study on the effects of PCP on the development of feeding behaviour over the first eight weeks of life (to be reported on elsewhere) were photographed and total lengths measured from the photographs. For photography the fry were confined in a narrow (1.0 cm) water-filled space between two sheets of glass which included a millimeter rule. Measurements were made on only the fry in focus and lying straight. At the commencement of the PCP exposures, based on a sample of 10 fish from each of the six exposure concentrations, there were no

significant differences in the lengths of the fish of the different treatments (overall mean 5.6 mm, SD 0.7; one-way ANOVA, $F = 0.14$, $p>0.716$, d.f. 5,54).

RESULTS AND DISCUSSION

Food conversion efficiency (FCE) was significantly affected in a concentration-dependant fashion at concentrations above 10 µg/ L (ANOVA $F = 10.3$, $p<0.0001$, d.f. 5,46; Fig. 1a). The relationship is described by the equation:

$$FCE\ (g/g) = 0.07 - 0.04\ \ PCP\ (\mu g/L)\ \ ;\ n = 47,\ r^2 = 0.470,\ p>0.0001.$$

The threshold for a PCP effect on conversion efficiency can be determined from the intercept between the zero response line and the concentration response regression line (Webb and Brett 1973). The threshold effect level in this experiment is 23.4 µg/L.

A plot of the total length data for 52 day old fry exposed continuously from day one to a comparable range of PCP concentrations results in a plot (Fig. 1b) similar in shape to that of the FCE data. With exposure to increasing PCP concentrations above 10 µg/L, there was a significant concentration related reduction in total length (TL) (ANOVA $F = 36.5$, $p<0.0001$, d.f. 5,209). The equation describing the relationship is:

$$TL\ (mm) = 22.1 - 0.042\ \ PCP\ (\mu g/L)\ \ ;\ n = 210,\ r^2 = 0.429,\ p>0.001.$$

For the length data the threshold response value is 25.2 µg/L which is very close to that of the FCE value (23.4 ug/L). These threshold values are within the range, 2.0 to 100 µg/L, reported by others to affect growth of several non-centrarchid fish species (Kruger et al. 1966, Webb and Brett 1973, Hodson and Blunt 1981, Holcombe et al. 1982, Sloof and Canton 1983). The response threshold of 25 µg/L at which growth is retarded is greater than the US-EPA criterion level of 48 µg/L (Yount and Richter 1986); at this latter concentration food conversion efficiency in our study was reduced by approximately 25%.

In our other studies (Johansen et al. 1985) on the effects of PCP on young bass we found that the 96 h LC50 for fish up to 4-5 weeks of age was 285 µg/L. This declined to a value of 159 µg/L by seven weeks and remained at this level for bass two years of age. A 120 day LC50 was estimated at 54 µg/L. The threshold concentration resulting in a decline in feeding activity of bass over their first eight weeks of life was 45 µg/L (28% of the 96 h LC50) (Brown et al. to be reported elsewhere). The threshold level for reducing certain components of predator avoidance behaviour of guppies (Poecilia reticulata) was 50% of the 96 h LC50 (Brown et al. 1985). Thus, when comparing PCP induced mortality, behavioural responses, length at the end of a long term

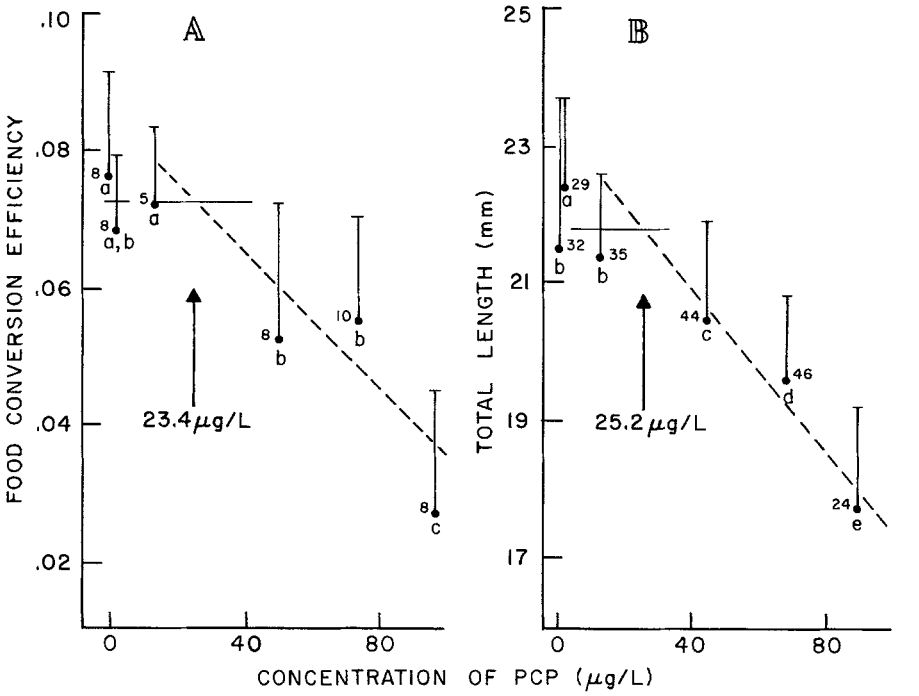

Figure 1: (a) Food conversion efficiency as g (growth)/ g (food) in a seven day experiment, and (b) total length (mm) of largemouth bass at the end of a 52 day exposure to several PCP concentrations. Within each experiment means (s.d.) with the same letter are not significantly different ($p > 0.05$). The number beside each point is the sample size. The intercept of the no-response line (solid) and the PCP-response line (dashed) estimates the threshold of response (indicated by the arrow).

exposure and food conversion efficiency ; the latter two are the most sensitive indicators of PCP effects on fish for they are affected at 16% of the 96 h LC50. It also appears that short term experiments monitoring food conversion efficiency are effective in assessing the effects of PCP in that they give similar results to long term studies. For a general metabolic poison with a primary effect on energy metabolism it is not surprising that growth is a sensitive indicator. However, it is anitcipated that, for toxic agents with other primary sites of action such as the central nervous system, other responses, like altered behavioural activity, may prove to be the most sensitive indicator for threshold effect levels. It is obvious that biological tests aiming at determining acceptable limits of toxic agents in the environment must focus on the most sensitive biological processes linked to the mode of action of the agent in question.

The ecological significance of reduction in growth rate is often uncertain (Woltering 1984). However, it has been shown that

survival of young-of-year bass is strongly influenced by their growth rate. Hunter (1981) pointed out that predation is heavier on smaller individuals; also, Oliver et al. (1979) and Toneys and Coble (1979) found a reduction in overwinter survival correlated with small body size in bass. Therefore, PCP induced growth reduction could have an effect on the winter survival of young-of-year bass. This apsect of the problem warrants investigation.

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