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Mortality of Early Life Stages of Largemouth Bass, *Micropterus salmoides* due to Pentachlorophenol Exposure

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Pentachlorophenol (PCP), a biocide used frequently in the wood preservation industry, is present at detectable levels in many bodies of freshwater (Jones 1981). Because of its wide use and the possibility of contamination of non-target organisms much is known about its chemistry, pharmacology, and toxicology (see Rao 1978). A review of fish life-cycle toxicity tests suggests that embryo-larval and early juvenile stages are among the most sensitive for most toxicants (McKim 1977). However, in this respect less seems to be known about the effects of PCP on the early life stages of fish although Borthwick and Schimmel (1978) have reported on 96 h LC50 studies on some estuarine animals, while Chapman and Shumway (1978) and Hodson and Blunt (1981) have studied the effects of PCP on the developmental and early alevin stages of Salmo gairdneri. The latter two studies demonstrate the toxic nature of PCP to these early life stages of a cold water fish. In this paper we report on the toxicity of PCP to various life-cycle stages of a warm water species, the largemouth bass (Micropterus salmoides). Also, using long term exposures to low concentrations of PCP, we determine the highest PCP concentration which causes no significant increase in mortality.

MATERIALS AND METHODS

Acute toxicity tests, in the form of 96 h LC50 determinations, were carried out in static water that was changed daily while chronic toxicity tests were made using a continuous flow proportional dilution apparatus.

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

Fry in the holding aquaria were fed 3 times daily on live zooplankton from fertilized rearing pools and live brine shrimp

nauplii. Experimental fry were fed only brine shrimp nauplii 3 times daily. Frozen brine shrimp were provided once the fry were large enough to eat them. All holding and experimental containers were cleared of accumulated debris at frequent intervals. The 100 L holding aquaria were made of grey-painted wood with one glass side. All holding aquaria were supplied with continuously flowing lake water (alkalinity 65 mg/L as $CaCO_3$, Smol 1983; pH = 7.7) and a natural photoperiod, whereas the aquaria at the main campus laboratory were kept under a 12 h light - 12 h dark photoperiod and received dechlorinated municipal water (alkalinity 67 mg/L (as $CaCO_3$) and pH 7.2) at 25°C.

To prepare the concentrated PCP stock solution 500 mg of reagent grade pentachlorophenol (lot no. 102F-0044, Sigma Chemical Co., St. Louis, MO. U.S.A.; purity 99% by G.C.; contaminants not identified) was dissolved in 10.0 ml of 1.0 N NaOH solution which was diluted with distilled water to a total volume of 1.0 L.

The 96 h LC50 determinations were carried out in static PCP-treated water that was aerated and changed daily. The ratio of fish to water volume was kept at 120 mm total fish length per 1.0 L of test solution. At least 10 individuals were tested at each concentration. The fry selected for the LC50 trials were reared and tested at 25°C. LC50 trials were carried out periodically over the summer (see Table 1) to test for changes in the LC50 as fry grew older and larger. Throughout a trial the containers were checked frequently and dead individuals removed and measured. The temperature of 25°C was chosen as a constant test temperature since it is well below the upper lethal temperature and near the temperature optimum (27°C) for growth of bass fry (Straun 1961).

To achieve chronic PCP exposure, fish were held in drain-equipped 20 L plastic pails that received a continuous 100 ml/min flow of PCP treated or untreated water from a proportional diluter. Each pail was aerated continuously. The diluter consisted of two constant head manifolds that received temperature regulated water at a constant flow rate and aeration. To one of the manifolds was added PCP stock solution by a peristaltic pump. By drawing different water flow-rates from each manifold into common mixing funnels prior to distribution to the pails holding the fish, very stable exposure concentrations were achieved. In this fashion 3 replicates of the desired exposure concentrations of untreated water, 0.03 $\pm$ 0.01, 0.9 $\pm$ 0.4, 7.5 $\pm$ 1.7, 41.0 $\pm$ 7.7 and 83.0 $\pm$ 13.8 µg PCP/L were achieved. The above values represent mean $\pm$ standard deviations of daily calculated PCP concentrations over the course of several weeks. These concentrations were chosen to span the range of PCP levels that might be expected in the environment (Jones 1981). The actual concentrations were calculated from measured daily flow rates from the manifolds into the mixing funnels and the flow of the PCP stock concentrate. Periodic water analysis for PCP using electron capture GLC confirmed the accuracy of the calculated concentrations for the day of sampling. Chronic exposure of bass fry to the above

concentrations began on day 1 of free-swimming and continued for 120 days with only one interruption. Ninety fry per exposure concentration were distributed evenly amongst the 3 pails at that concentration. While at the field station precise temperature control was not possible but the water supply was regulated so as not to fall below 18°C at night. Day time temperatures (mean of 23.8 ± 2.3°C) over this period ranged up to 27°C.

The fish chronically exposed to PCP were for studies of feeding behaviour to be reported elsewhere but over the course of the experiment measurements of total length and survivorship were made biweekly, and the distribution amongst the 3 pails at each concentration equalized.

On day 36 of PCP exposure some fish were noticed to be mildly infected with ich (Ichthyophthirius multifiliis). PCP dosing was discontinued and, on a random selection of 5 fish from each concentration, counts were made of the white ich spots. All fish at all concentrations were treated with "Contra Ick 80" (Tetrawerke, W. Germany). By day 39 there was no sign of the infection, which did not recur, nor was there any indication that the infection caused any mortality. On day 49 after the move to the main campus dosing was resumed and continued uninterrupted to day 120.

The 96 h LC50 data were analyzed using the GLIM statistical package and Chi-square comparison (Royal Statistical Society, London, 1977).

RESULTS AND DISCUSSION

The 96 h LC50 data for fry up to 84 days of age are summarized in Table 1. All possible pairs of age combinations were subjected to Chi-square analysis. The LC50 data for fry at 14 and 28 days do not differ ($\chi^2 = 0.096$, $p > 0.70$) (combined 96 H LC50 = 285 µg/L) nor do the LC50 data for 49 and 84 day old fry ($\chi^2 = 0.667$, $p > 0.30$) (combined 96 h LC50 = 159 µg/L). All other LC50 combinations do differ significantly ($\chi^2 \geq 13.0$, $p < 0.01$). We therefore concluded that between 28 and 49 days of age largemouth bass fry become significantly less tolerant to PCP poisoning.

The 96 h LC50 of 1+ and 2+ years old juvenile bass were also determined but for only a limited number of fish (5 fish of each age group at 4 concentrations). For both age groups the LC50 value was 194 µg/L, not dissimilar from that of bass at 49 and 84 days of age. Further statistical analysis was not possible due to the small sample size.

Our 96 h LC50 results for bass fry are in line with values reported for other fish like Rasbora (Gupta 1983), Pimephales promelas (Cleveland et al. 1982), Oncorhynchus tschawytscha (Iwana and Greer 1978) as well as for several other fish species (Slooff et al. 1983; Jones 1981).

A similar shift in PCP tolerance for juvenile sheephead minnow

Table 1. Results of 96 h LC50 determinations on juvenile largemouth bass. Pairwise chi-square analyses were made on all age combinations; pairs not sharing the same letter are significantly different ($p < 0.05$).

Age (days)	96 h LC50(µg/L) (95% confidence limits)		length (mm) ($\bar{X} \pm$ s. dev.)
14	287 (239-344)	A	8.1 ± 1.0
28	275 (221-341)	A	11.3 ± 1.8
49	136 (105-177)	B	22.2 ± 1.7
84	189 (105-340)	B	55.8 ± 3.3

(Cyprinodon variegatus) was found by Borthwick and Shimmel (1978). However, they did not comment on this or even state whether the differences were statistically significant. As well similar losses of tolerance have been reported for toxic substances in other fish species: arsenic in Esox musquinongy (Spotila and Paladina 1979), nonyl phenol ethoxylate in Salmo gairdneri (Marchetti 1965), and chlorine in Morone saxatilis (Hall et al. 1982). Of greater interest is the cause for the shift in tolerance to PCP.

PCP is a general metabolic poison which at low concentrations uncouples oxidative phosphorylation without retarding electron transport. At higher concentrations it reduces the activity of some glycolytic enzymes while increasing the activity of some enzymes of the HMP pathway and TCA cycle along with cytochrome oxidase (Bostrom and Johansson 1972). In a recent paper Forstner et al. (1983) showed that Coregonus sp. undergoes a "biochemical metamorphosis". For about the first 40 days following hatching key glycolytic enzymes have markedly reduced levels of activity which, after this time, rise to higher levels. During these same initial 40 days key TCA cycle enzymes as well as cytochrome oxidase have high or progressively increasing levels of activity. These results indicate that for the first 40 days lipid is the primary energy source, and that only later with the increase in activities of glycolytic enzymes is it possible for carbohydrate to make a significant energetic contribution. Thus one might postulate that bass also undergo a "metamorphosis" in some facet of energy metabolism at around 30 to 40 days of age which accounts for the reduction in PCP tolerance found at this same time period. Further speculation is not warranted, but

suggests a future line of inquiry in an attempt to understand this loss of tolerance.

Chronic exposure of bass to low concentrations of PCP over the first 120 days of life resulted in concentration related progressive mortality (Fig. 1). Multiple linear regression analysis of these data gives the regression equation:

$$Y = -1.11 + 32.90 \log \text{time} + 3.73 \log \text{concentration}$$

$$(R^2 = 0.94,\ p < 0.0001)$$

where Y is % mortality in arc sine transformation, time is in days, and PCP concentration in µg/L. Both time ($p < 0.0001$) and PCP concentration ($p < 0.0021$) contributed significantly to the mortality.

From the mortality data at 120 days of exposure we calculated a 120 day LC50 value of 54 µg/L. This LC50 value is roughly a third of the 96 h LC50 for fry 49-84 days of age.

Chi- square analysis of the mortality data (expressed as counts) at 120 days showed that the mortality of the 83 µg/L treatment was significantly higher than that of the untreated group ($\chi^2 = 21.98$, $p < 0.001$) whereas the other treatments, including 41 µg/L, were not significantly different from the untreated group ($\chi^2 \leq 2.65$, $p > 0.20$). The mortality experienced by the 0.03 µg/L treatment was lower (though not significantly lower) than the untreated group throughout the exposure period. Thus the maximum acceptable toxicant concentration (McKim 1977) should lie between the two values of 41 and 83 µg/L. PCP levels recorded in Great Lake waters do not approach the 41 µg/L level except for some waters near industrial effluents (Jones 1981).

These long term mortality experiments are complicated by the high mortality experienced by the untreated fish. A high early life stage mortality, up to 99% during the first 130 days of life, can be expected in fish (Bagenal and Braum 1978) with starvation and predation being major sources of mortality (Hunter 1981). As well the switch over to active feeding is a high risk period (May 1974). Exposure to PCP began prior to the beginning of feeding on shrimp nauplii. That large numbers of fry died due to not feeding at all or at an insufficient rate over the first several days or to other unrecognized causes is quite possible given the above mentioned studies.

A second complication was the necessity to discontinue dosing on day 36 and treat the fish for an ich infection for 3 days, then leave the fish unexposed to PCP for a further 10 days before resuming the experiment in a new laboratory setting on our main campus. The water chemistry in terms of hardness and pH was not much different from that at the Field Station. As stated previously there was no undue mortality that could be attributed to the infection which was treated in its earliest stages or to

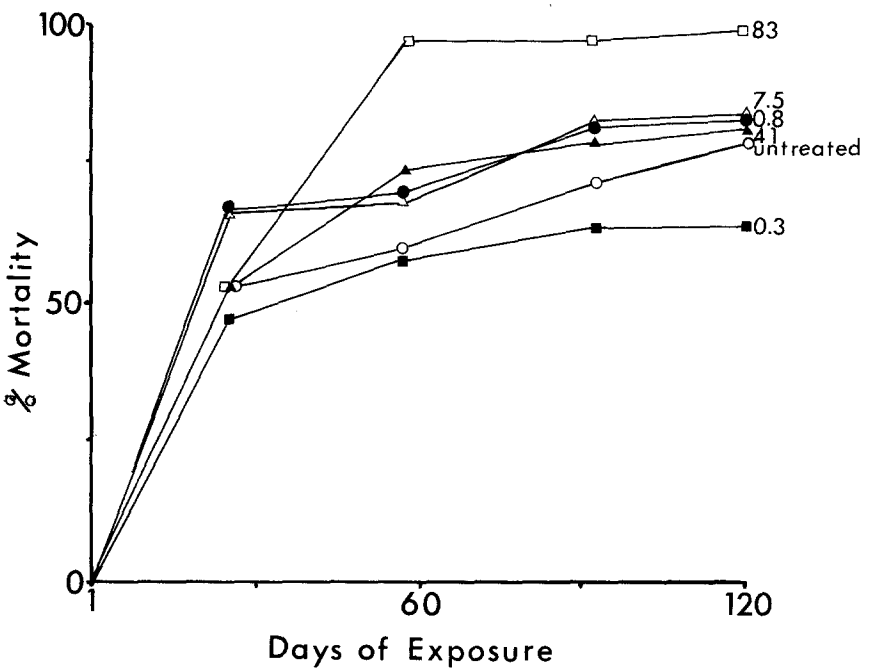

Figure 1: Percent mortality over 120 days of exposure for treatments exposed to various concentrations of PCP (shown next to line, in µg/L).

the change of location. In a field situation young fish have to contend with many factors such as infection, relative food abundance, etc. to which, in many areas, has been added exotic toxic chemicals of which PCP is an example. Though the absolute 120 day LC50 may be imprecise nonetheless the chronic exposure experiment demonstrates the additive effect of a chemical present in concentrations well below acutely toxic levels on natural or expected early life stage mortality.

The weekly length measurements of the first 5 weeks of PCP exposure were subjected to regression analysis and it was found that the slightly reduced growth at the 3 higher PCP concentrations was not significant. This experiment was not designed specifically to monitor growth but this result is surprising because PCP has been found to decrease the growth of other fish species (Hodson and Blunt 1981). We are currently experimenting to determine the joint effects of food ration and PCP exposure on the growth of fish. The ich infection was most severe on the fish from 83 µg/L concentration with over 102 white spots in total while all others, including the controls, ranged between 32 and 0 though not in a dose related fashion. It would seem then that the highest concentration was definitely imposing a physiological load making the fish more susceptible to infection.

In conclusion largemouth bass fry under 30 days old are more tolerant of PCP poisoning than older bass, and prolonged exposure to concentrations well below the 96 h LC50 value significantly increases mortality.

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