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Acute and Chronic Toxicity of Sodium Pentachlorophenate to the Copepod, *Pseudodiaptomus coronatus*

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Pentachlorophenol (PCP) and its sodium salt, sodium pentachlorophenate (Na-PCP) have many diverse applications in industry and agriculture. Together, these are the second heaviest-used pesticides in the United States (CIRELLI 1978). Na-PCP is highly soluble in water, depending upon pH (BEVENUE & BECKMAN 1967) and therefore poses a threat as a contaminant to waterways and estuaries via runoff from agricultural areas, industrial wastes (BEVENUE & BECKMAN 1967), municipal sewage (BUHLER et al. 1973), and industrial accidents (PIERCE 1978).

The major biocidal activity of this compound is considered to be the inhibition of oxidative phosphorylation (WEINBACK & GARBUS 1969) but it affects other physiological mechanisms as well (BEVENUE & BECKMAN 1967, SAARIKOSKI & KAILA 1977, FOX & RAO 1978). Although numerous studies have been made on the toxicity of PCP to terrestrial and freshwater organisms relatively little is known about its effects on marine or estuarine organisms, especially zooplankton. Some studies have been made of pelagic larvae of nektonic and benthic animals (VAN DIJK et al. 1977, BORTHWICK & SCHIMMEL 1978); however, evidence demonstrating that adult marine zooplankters experience similar sensitivity to Na-PCP as do planktonic larvae of benthic animals is lacking. For this reason, this study was undertaken to examine the acute toxicity of Na-PCP to adults of the copepod Pseudodiaptomus coronatus, and to determine chronic toxicity on the basis of ingestion rate.

P. coronatus Williams, a neritic calanoid copepod, is distributed along the coast of North America from Nova Scotia to the Mississippi River delta region (WILLEY 1923, WOODMANSEE 1958, DEEVEY 1960, GRICE 1960, CUZON DU REST 1963; as cited in GRICE 1969). Although this species is not usually dominant, it has been found to dominate at times (JACOBS 1961). Also, it has been shown, in at least one region, to be the main

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food organism of the fringed flounder, Etropus crossotus (STICKNEY et al. 1974).

MATERIAL AND METHODS

The copepods were captured in the Indian River, Brevard Co., FL and were cultivated in artificial seawater to eliminate any interference which may have been caused by previous exposure to PCP. Acute toxicity to Na-PCP was determined by performing a static bioassay (AMERICAN PUBLIC HEALTH ASSOCIATION 1976). The tests were performed in 150 ml borosilicate glass beakers, each containing 100 ml of the toxicant solution and 20 copepods. The medium used was the artificial seawater formulation of GENTILE & SOSNOWSKI (1978) adjusted to a salinity of 18°/oo with the temperature maintained at 20°C (±4°C). Dose-mortality data were analyzed according to the method of LITCHFIELD & WILCOXON (1949).

P. coronatus is incapable of surviving the 96-hr test period without food, therefore, the animals were provided with a daily diet of approximately 3.3×10^6 cells of Thalassiosira pseudonana and 2.7×10^6 cells of Isochrysis galbana.

Chronic toxicity was observed as alteration of ingestion rate during the bioassay. This was determined indirectly by observing fecal pellet production, since a high correlation exists between fecal pellet production and ingestion rate (GAUDY 1974). Fecal pellets were counted daily, without harvesting, by placing each bioassay vessel over a grid and enumerating only those pellets within the borders. These data were reported as daily fecal pellet production per live animal calculated by subtracting the count of the previous day from the daily count and dividing this difference by the number of remaining copepods.

After the 96-hr bioassay, the PCP was extracted from the unfiltered medium according to the method of PIERCE (1978) and measured by high performance liquid chromatography (HPLC) using a Varian Model 5020 Liquid Chromatograph. The column was 30 cm x 4 mm packed with MicroPak MCH-5 (monomeric C_{18} bonded onto 5 silica gel) and a guard column packed with Vydac RP. Column temperature was 30°C, and the eluent was an isocratic mixture of 90% methanol and 10% water with 1% acetic acid at a flow rate of 1.0 ml per minute. The UV absorbance detector used operated at 254 nm and was capable of detecting PCP residues down to 3.0 ng.

RESULTS AND DISCUSSION

P. coronatus experienced acute toxicity to Na-PCP with a 96-hr LC50 value of 68.0 $\mu g \cdot l^{-1}$ and a 95% confidence interval of 32.6 - 141.8 $\mu g \cdot l^{-1}$ (Figure 1).

These concentrations are based on the amounts added initially, since subsequent extraction and measurement revealed a decline in PCP concentrations of approximately 80%. A duplication of the bioassay procedure (without copepods) showed that the decline followed this scheme: 59% decline after 12 hr; 60% after 24 hr; 71% after 72 hr; and 80% after 96 hr. As a result, the bioassay more closely simulated a PCP spill rather than a constant source such as an industrial effluent.

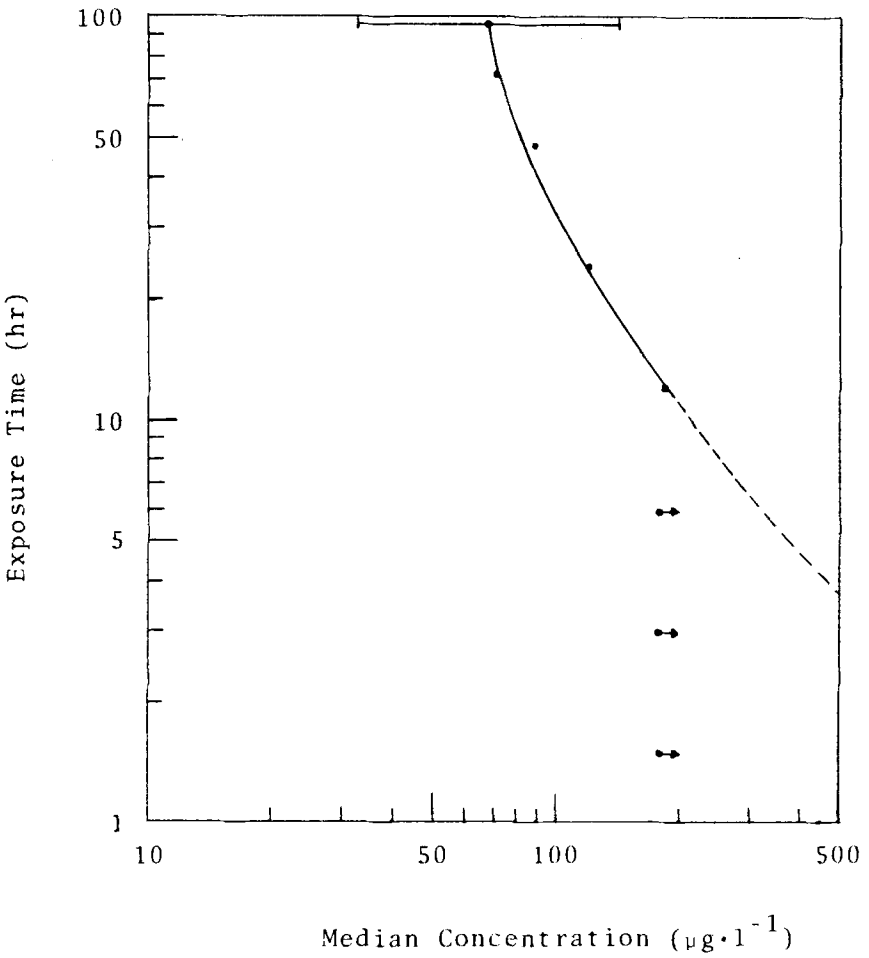

FIGURE 1. Toxicity curve. Drawn from LC50 values determined from dosage-mortality curves and straight-line interpolation of mortality data. The 95% confidence interval is shown for the 96-hr value.

For this reason, the 68.0 $\mu g \cdot l^{-1}$ LC50 represents a maximum value, since copepod mortality would probably have been higher had they been exposed to this concentration over a longer period of time.

The toxicity to this copepod appears to be quite high when compared with that of various adult marine and estuarine organisms. However, it does compare favorably with toxicity to some developmental stages which are similar in size to the copepod. Examples of the 96-hr LC50 values and (95% confidence intervals) are: the developing embryo of the eastern oyster, Crassostrea virginica, 40.0 $\mu g \cdot l^{-1}$ (36.0 - 44.0); the 48-hr prolarvae of the pinfish, Lagodon rhomboides, 38.0 $\mu g \cdot l^{-1}$ (26.0 - 57.0); and the first instar larval stage of the decapods Crangon crangon, 112.0 $\mu g \cdot l^{-1}$ (97.0 - 122.0) and Palaemon elegans, 84.0 $\mu g \cdot l^{-1}$ (27.0 - 125.0) (VAN DIJK et al. 1977, BORTHWICK & SCHIMMEL 1978).

The fecal pellet data show that exposure to lethal levels of Na-PCP resulted in an increase in feeding rate (Figure 2). Analysis of variance of these data indicated that generally there was no significant difference in feces production among the control, 18.0, 32.0, and 56.0 $\mu g \cdot l^{-1}$ Na-PCP. Organisms exposed to 81.0 $\mu g \cdot l^{-1}$, however, exhibited a significant increase in fecal pellet production rate, about 12 times that of the control at 96 hr.

The procedure used to quantify fecal pellet production was chosen because it involved a minimum of disturbance to the animals, an important consideration since this analysis was performed concurrently with the bioassay. A more sensitive method, however, must be utilized to determine sublethal effects on feeding rate. One such procedure would involve direct counts of algal cells as used by MULLIN 1963.

The observed increase in ingestion rate can be attributed to PCP inhibition of oxidative phosphorylation, which results in a decrease in ATP production. A decline in ATP levels can affect basic physiological mechanisms such as the Embden-Meyerhof pathway of glycolysis. Step three of this pathway is the phosphorylation of fructose-6-phosphate by the enzyme phosphofructokinase (PFKase) and is considered to be the major rate limiting reaction (LEHNINGER 1975). PFKase is an allosteric enzyme with binding sites for either ADP or ATP depending on the ADP/ATP ratio. A decrease in ATP results in a high ADP/ATP ratio, the enzyme binds with ADP and becomes fully active, glycolysis proceeds rapidly depleting glucose levels in the blood and thus triggering a hunger response.

CONCLUSION

The planktonic copepod, Pseudodiaptomus coronatus

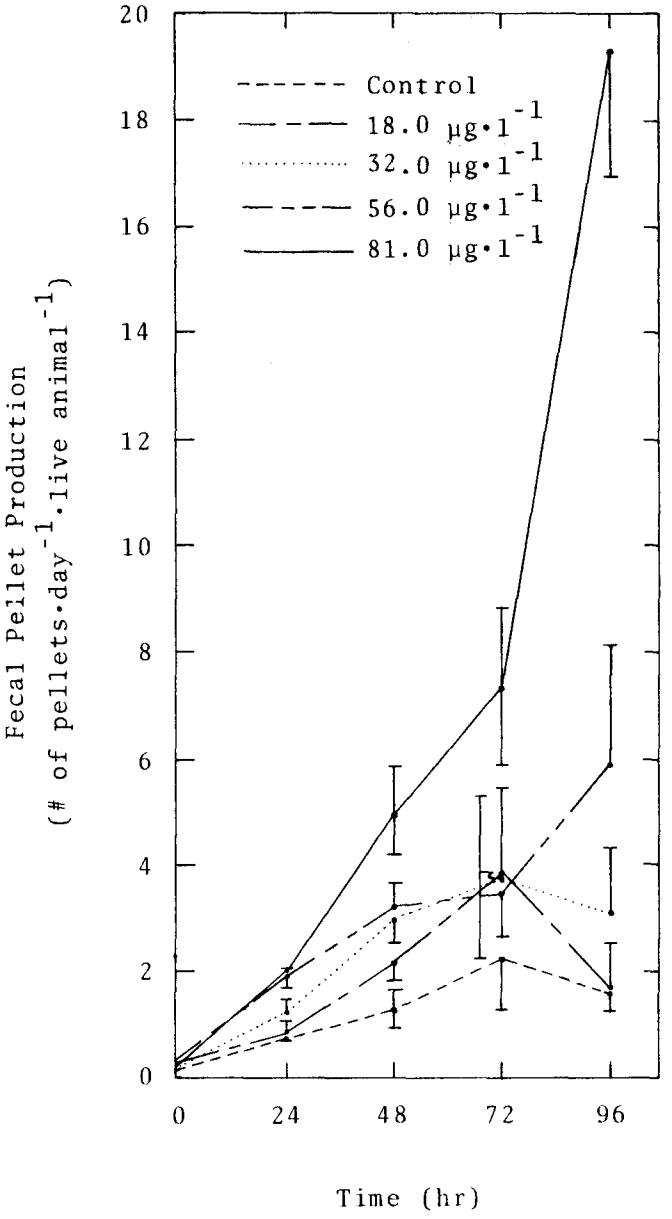

FIGURE 2. Relative fecal pellet production in different Na-PCP concentrations during bioassay.

experienced relatively high acute toxicity to Na-PCP with a 96-hr LC50 value of 68.0 $\mu g \cdot l^{-1}$ (based on the initial concentrations). This value compares favorably with meroplanktonic larvae of several large nektonic and benthic animals.

Chronic toxicity was demonstrated by an increase in fecal pellet production representative of ingestion rate. Exposure to Na-PCP resulted in an increase in feeding rate which may have resulted from PCP inhibition of oxidative phosphorylation. This would result in a decrease in ATP which alters glycolysis.

This work shows that concentrations of Na-PCP below 100 $\mu g \cdot l^{-1}$ could alter community structure by a two-step sequence. Overexploitation of primary producers would begin within 24 hr of exposure due to an increase in feeding rate of copepods. This overexploitation would continue until mortality of these animals predominates, resulting in a reduction of secondary producers as well.

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