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Effects of Sublethal Concentrations of Pentachlorophenol on the Liver of Bluegill Sunfish, *Lepomis macrochirus*

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Pentachlorophenol (PCP) and its salts, primarily sodium pentachlorophenate (SPCP), have been used extensively in industry and agriculture as fungicides, bactericides, and insecticides (CARSWELL & NASON 1938; HUECK & LA BRIHN 1960). Accumulation in fish storage tissues, eggs, and embryos can occur either by direct uptake from water, food, or a combination of both (HUNT 1966). Long term exposure to a low concentration may cause tissue damage (COUCH 1975) and also affect the reproductive potential of certain fish species (RUDLING 1970; COTE 1972; and PIERCE et al. 1977).

This study was designed to complement the research of PRUITT et al. (1977) on accumulation and elimination of sublethal concentrations of PCP (0.1 ppm) in bluegill sunfish. It was felt that this offered an excellent opportunity to histologically monitor a specific fish organ in which sublethal PCP was being measured. The liver was selected because PRUITT'S preliminary experiments showed that it accumulated higher concentrations of PCP (35 µg/g) than did muscle tissue or other organs. Their preliminary observations also indicated that sunfish exposed to PCP would not feed consistently; therefore, we conducted, as did PRUITT, exposure experiments in the absence of food. We also found it necessary to histologically differentiate the effects of starvation by incorporating starvation experiments on unexposed fish maintained under similar conditions as those exposed to PCP.

MATERIALS AND METHODS

Six month old male and female bluegills, ranging from 16 g to 25 g, were obtained from the Mississippi Game and Fish Commission Hatchery, Lyman, Mississippi, and the Meridian National Fish Hatchery, Meridian, Mississippi. They were acclimated to laboratory conditions in 150 gallon fiberglass aquaria and stabilized against infection by periodic exposure to 1.5 ppm acriflavine hydrochloride over a 2 week interval. Bluegills were then randomly divided into control, starvation, and exposure groups. Control fish were fed daily; those fish used in the starvation and exposure experiments were not fed for 24 hours prior to and for the duration of the experiments. Two specimens were removed daily from the aquaria of each group on days 1, 2, 4, 8, 16, and 32 for histological examination. The 0.1 mg/liter PCP solution used in the PCP experiment was prepared by diluting 1 g PCP in 1 liter of acetone

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and further diluted with dechlorinated tap water to 0.1 g/liter (PRUITT et al. 1977). The control and starvation aquaria received the same amount of acetone as the PCP test solution. Aquaria were monitored for pH (7.2 - 7.7), temperature (17.0 - 21.0 C) and dissolved oxygen (7.4 - 8.6 mg/liter). Pentachlorophenol content was measured and verified after the initial 24 hours; test solutions were changed daily to compensate for any possible PCP loss.

For histological examination entire livers were excised quickly and placed in either Bouins' fixative or buffered formalin for 24 hours (HUMASON 1972). After prescribed washing and dehydrating procedures, tissue was cleared in xylene and embedded in paraffin. Tissue sections were stained in Delafield's hematoxylin and counter-stained with eosin dissolved in 95% alcohol. After dehydration and clearing, tissues were mounted in pine resin (ROSSO & BLAKESLEE, unpublished).

Glycogen was determined by fixing liver in cold ethanol-formalin, dehydrating, clearing, embedding in paraffin, and treating sectioned tissue with periodic acid and Schiff reagent (PAS technique; HUMASON 1972). Complementary sections were incubated in human saliva for 20 minutes and then treated with PAS. Lipids were confirmed from frozen sections processed for staining with either Oil Red O or Sudan IV solutions and then counterstained with hematoxylin (Manual of AFIP, 1968).

Hall's method for bilirubin and Perl's method for iron were used to assay the yellowish-brown pigment characteristic of bluegill macrophage cells (Manual of AFIP, 1968).

RESULTS

The liver of Lepomis macrochirus, as in many other teleosts, lacks the distinct lobules usually characteristic of higher vertebrates. Hepatic sheets or cords, usually 2 cells in thickness, are interconnected with similar sheets and are arranged around central veins in a radial, net-like pattern. Sinusoidal spaces perforate these plates and anastomose with other sinusoids (Fig. 1). Irregular bands of densely basophilic acinar pancreatic tissue lie adjacent to branches of the portal veins and bile ducts within certain portions of the liver (Fig. 4).

Controls. Livers of bluegills maintained under control conditions were examined following days 1, 2, 4, 8, 16, and 32. Peripheral and central hepatocytes had distinct cell boundaries and stained similarly with hemotoxylin and eosin. Hepatocytes retained the plate-like orientation throughout the experiment. Cytochemical differentiation with PAS reagent indicated that glycogen reserves were retained throughout the experiment. Nuclei, which measured approximately 4 μm in diameter, lacked the prominent nucleoli typical of PCP-exposed bluegills; hepatocytes measured approximately 11 μm through their greatest dimension. An occasional control fish

had aggregations of macrophages in the vicinity of smaller veins. These cells appeared yellowish-brown following H & E staining; after treatment with Perls', their blue color indicated that the pigment contained ferric iron.

Starvation Experiment. Livers of fish starved for only 1 day exhibited glycogen loss, cytoplasmic shrinkage, and nuclear crowding. These conditions also continued through day 2. By the end of day 4, small vacuolated areas with distinct boundaries became conspicuous in the relatively dense cytoplasm. After day 8, lipid was confirmed in the hepatocyte vacuoles, indicating the initiation of fatty change (GONZALEZ, personal communication, 1980). Certain peripheral hepatocytes lacked affinity for both nuclear and cytoplasmic stains, although other peripheral cells and most centrally-located cells stained normally. Peripheral cells, especially those adjacent to veins, apparently had more lipid accumulation than centrally-located cells.

After 32 days of starvation, hepatocytes had distinct lipid accumulations; however, nuclei were still located near the center of the cells (Fig. 2). Throughout the starvation interval, the sheet-arrangement of hepatocytes remained unaltered. Aggregations of vein-related macrophages containing iron-positive pigment were only occasionally observed.

Exposure Experiment. After 1 day of exposure to 0.1 mg/liter PCP, the liver still retained the normal sheet-type arrangement of hepatocytes, most of which contained notable glycogen reserves. The most obvious changes, however, were increased numbers of nuclei with prominent nucleoli, an apparent increase in iron-positive macrophages in the vicinity of veins, and a tendency of some peripheral hepatocytes to become eosinophilic and for cell boundaries to become less distinct. In specimens exposed for 2 days, peripheral eosinophilia was more accentuated and peripheral nuclei were basophilic, whereas centrally located cells had hypochromic nuclei with distinct nucleoli. Glycogen was absent from practically all cells and cell boundaries were almost indistinguishable. Groups of cells, apparently multikaryotic, disrupted the cord-like organization of the hepatocytes (Fig. 3).

After 4 days the exposed livers resembled a more normal liver than did livers of fish exposed for 2 days. There was less clumping of cells, fewer paired nuclei, and an apparent tendency for hepatocytes to reorganize into a sheet-type system. Iron-positive macrophages were still obvious; peripheral and central cells had similar staining affinities for H & E. At the end of 8 days of exposure the overall liver organization was somewhat similar to that of the 4 day exposure interval; however, cytoplasmic dissociation and cellular hypertrophy were common, indicating that portions of the liver were undergoing piecemeal necrosis. By the end of the 16th day of exposure, a normal cord-like arrangement of hepatocytes occurred in various parts of the liver; other areas still exhibited

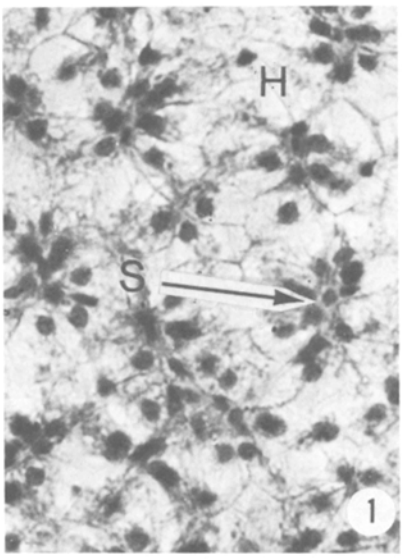
foci of dying and regenerating cells. Aggregates of iron-positive macrophages were associated with most of the veins in any given tissue section. After 32 days of exposure, the entire livers were pale yellow when dissected, indicating a "fatty" condition. Lipid globules, apparently larger than those of specimens exposed for 16 days, occupied much of the cell volume. Lipid accumulation, however, had not proceeded to the point of displacing the nuclei from the cell center in most hepatocytes. Although there were liver areas with well organized hepatic cords, the overall organization of most liver hepatocytes had not improved over that of the 16 day exposure specimens.

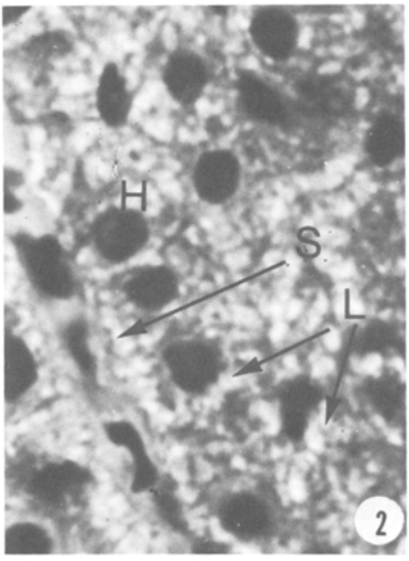
DISCUSSION

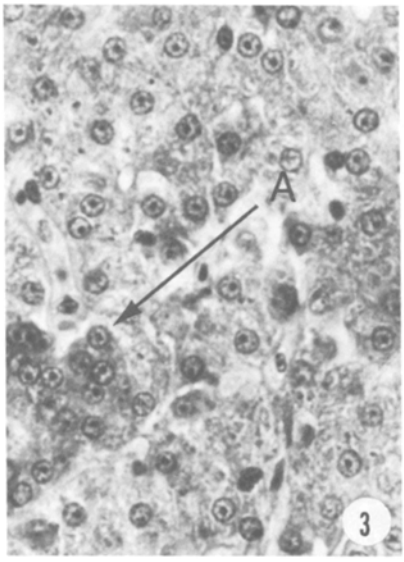
Glycogen loss, cytoplasmic shrinkage, nuclear crowding, and lipid accumulation were the conspicuous cytological changes common to livers of starved unexposed and starved-PCP exposed bluegills. Glycogen loss occurred after only one day in starved unexposed fish and after 2 days in exposed fish, followed by cytoplasmic shrinkage and nuclear crowding. Early glycogen depletion has been reported for various fish species upon food withdrawal or initiation of stress, including that of pesticide exposure (COUCH 1975). Lipid accumulation was observed after 4 days in starved unexposed bluegills, but was not observed until after day 32 in the exposed group. The delay in lipid accumulation in exposed fish may have been caused by a PCP-initiated increase in metabolism. HANES et al. (1972) reported that young Coho salmon catabolized 47% of total lipids in 14 days exposure to 0.1 ppm K-PCP, while control fish catabolized only 25%. HOLMBERG et al. (1972) also noted a relationship between lipid utilization and PCP exposure in eels treated with a 0.1 ppm solution.

Most of the degenerative changes induced by sublethal PCP, including hepatocyte cord disorientation, multikaryotic cells, pyknotic nuclei, and eosinophilia, occurred after only 2 days of exposure. The overall improvement shown by increased cord orientation and a decrease in the cellular effects by day 4 suggests a relatively rapid rate of cell turnover and an ability to adjust to PCP (GONZALEZ 1980). Hepatic cord organization apparently improved more between exposure day 4 and 16 than in the experimental interval between day 16 and 32. Numerous iron-positive macrophages, which appeared during the first stages of exposure, were consistently found in clusters adjacent to hepatic veins. Control and unexposed bluegill liver sections usually showed only one or two macrophage clusters adjacent to veins. The multinucleated cells observed after the 2 day exposure interval (Fig. 3) may be proliferated bile duct epithelial cells.

CRANDALL & GOODNIGHT (1963) observed a "coagulated" liver with few sinusoids in guppies (Lebistes reticulatus) treated with 0.5 ppm Na-PCP at pH 8.4 - 8.6 for over 180 days. As CRANDALL & GOODNIGHT explained in their report, a high pH was used in order to







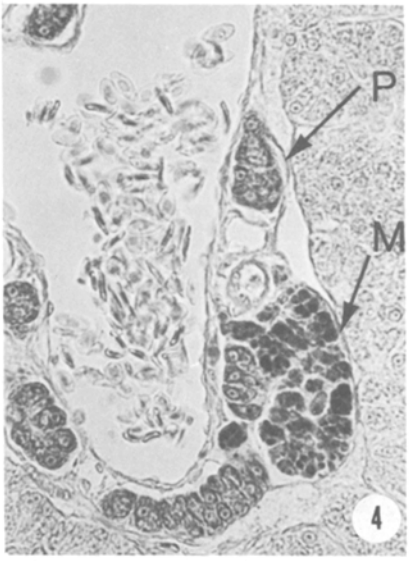


Figure 1. Control liver showing hepatocytes (H) and sinusoids (S) in cross-sectional view. H & E. X 800. Figure 2. Liver of fish starved for 32 days. H & E. X 1900. (S. Sinusoid; H. Hepatocyte; L. Lipid). Figure 3. Liver of fish exposed to PCP for 2 days with aggregates of unorganized cells (A). H & E. X 700. Figure 4. Pancreatic tissue (P) and macrophages (M) adjacent to vein in liver from a bluegill exposed for 16 days. X 440. Ferric iron in macrophages is indicated by treatment with Perls' method.

lessen the toxicity of their treatment solutions. Their experiments also emphasized long-term effects, whereas our study concerned short-term responses to PCP. In pond bluegills exposed to single applications of 1, 5, and 10 ppm 2,4-D, COPE et al. (1970) observed liver shrinkage and loss of vacuolation caused by glycogen depletion within 24 hours. After 28 days, morphological changes including bizarre parenchymal cells, distorted radial cords, and variations in staining intensity were evident. After 56 days, these lesions were commonly observed in fish treated with 10 ppm, but were rare in the 5 ppm samples. After 84 days only occasional bluegills still possessed lesions. In other studies concerning bluegills, hepatocyte cord disorientation and shrinkage were also induced by heptachlor (ANDREWS et al. 1966), methoxychlor (KENNEDY et al. 1970), and Abate (ELLER 1971a). Nuclear pyknosis and focal necrosis were also reported for bluegills exposed to dichlorobenil (COPE et al. 1969). KENNEDY et al. (1970) also reported eosinophilic masses in bluegill livers responding to methoxychlor treatment. Similar lesions have been induced in cutthroat trout by endrin (ELLER 1971b), in lake trout by Aroclor 1248 (ELLER 1971a), and in spot and pinfish by Aroclor 1254 (HANSEN et al. 1971).

In correlating our morphological observations with PCP accumulation data determined for bluegill liver by gas chromatographic analyses (PRUITT et al. 1977), it should be noted that improvement in liver organization following day 4 of exposure occurred during continued PCP accumulation. Their data showed an increase of PCP in the liver to 35 µg/g through day 8 followed by a decrease to 0.6 µg/g through day 16. PRUITT et al. noted that the decrease to 0.6 µg/g after day 8 was possibly related to the time required to develop an enzyme-related elimination or detoxification mechanism. Since they did not expose specimens for more than 16 days, we were unable to correlate lipid deposition noted in our 32 day specimens with a known PCP level.

It is evident that the lesions found in liver from bluegills exposed to sublethal PCP are not specific effects of PCP. Such lesions commonly occur in many fish species in reaction to various pesticides. The presence of these lesions, however, would serve to alert an investigator to the presence of a pesticide in the aquatic environment.

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