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Absorption of Dietary Dieldrin by Striped Bass

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The organochlorine pesticide dieldrin (3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth [2,3-b] oxirene), a cyclodiene chemical, has been restricted and banned in the U.S. since the early 1970's. Nevertheless, recent monitoring programs have shown that dieldrin still persists in the sediments and tissues of aquatic organisms (Summers et al. 1992). Dieldrin has a high potential to accumulate in fish because it has a low vapor pressure, adsorbs to soil, is lipophilic, and slowly metabolized in animal tissues (Grzenda et al. 1971; Sundershan and Khan 1981; Shubat and Curtis 1986; Gilroy et al. 1993). Dieldrin is a commonly measured environmental contaminant which is accumulated by fish and may be an indicator of contamination due to its stability and relative ease of measurement. In fish, ingestion of water and feed are the primary routes of dieldrin exposure. There remains some debate among researchers as to which pathway has greater influence on dieldrin accumulation (Chadwick and Brocksen 1969; Shannon 1977).

The possibility for dietary exposure to dieldrin by fishes in the wild is documented (Schmitt et al. 1981), yet information concerning the accumulation by oral route for most species is not available. The striped bass (Morone saxatilis), a popular commercially important fish in the U. S., was chosen for this study because there is limited dieldrin accumulation data for this species (Hayes 1974). Yet, the largemouth bass (Micropterus salmoides), which is not a relative of the striped bass but has similar feeding habits, has proven to be sensitive to dieldrin (Johnson and Finley 1980). The objective was to investigate the accumulation of dieldrin in the striped bass from treated food.

MATERIALS AND METHODS

Diets were manufactured at the Bozeman Fish Technology Center (U.S. Fish and Wildlife Service, Bozeman, MT). The open-formula diet, Striped Bass Grower

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9301 (SBG 9301), was used as the base diet for the feeding study. Six experimental diets were manufactured by adding dieldrin to SBG 9301 to obtain final concentrations of 0, 0.01, 0.10, 1.0, 10.0, and 25.0 mg/kg feed.

All ingredients were passed through a 20 mesh (U.S. Screen Size) sieve. After all the dry ingredients were well mixed, the herring oil containing the appropriate dieldrin level was added to the dry mix. Dieldrin (85% active ingredient, Sigma Chemical Co., St. Louis, MO) was dissolved in 50 mL of hexane (Ultra-Resi Analyzed, J.T. Baker) and mixed with herring oil. Each of the six diet formulations (5 kg) contained a total of 50 mL of hexane. Approximately 34% moisture was added to the mash prior to extrusion. The diets were manufactured using a cold extruder (model EXDC(F)S-60, LCI Inc., Charlotte, NC) and marumerizer (model QJ-400, LCI Inc.). After processing, the diets were dried in an ambient temperature, forced-air drier until less than 10% moisture remained in the feed (approximately 12 hr). The control and dieldrin contaminated feeds were analyzed for dieldrin (as described below) and found to be within 10% of the desired levels for dieldrin. Because the limit of detection for dieldrin was 10 ppb, dieldrin in the 10 ppb diet was not detected using the analytical method applied in this research. The pelletized food mixture, containing 10.5% lipid, was frozen and fed to the fish twice per day.

Fingerling striped bass of mixed sexes measuring 2.5-5.0 cm were obtained from BoGinn National Fish Hatchery (Millen, GA). Fish were raised about 6 mon on a commercial diet (Ziegler Salmon Starter).

For the first study, six groups of 40 striped bass weighing 18-22 g per fish were divided and placed into two separate 90% recycling systems. Each system (3660 L) consisted of a head tank which contained biofilters and three 900 L tanks which were partitioned by flow-through dividers to give six tank sections per system (Fig. 1). The water was aerated, maintained between 18 and 22°C, and between pH 6.8 and 8.0. Nitrite was maintained below 0.02 ppm and ammonia was maintained below 0.2 ppm. Prior to use, water was dechlorinated and passed through charcoal and biofilters before entering the tanks. Ten percent of the water was constantly released. Sixteen fish from each of the six experimental groups were chosen at random at 6 and 12 wk, sacrificed and weighed. The feed rate was 0.42% of fish body weight per day for the 6 wk period and 0.52% for the 6 to 12 wk period. Estimated total feed for the fish collected at 6 and 12 wk were 13.3 and 32.9 g feed per fish, respectively.

For the second study, six groups of 24 striped bass weighing 18-22 g per fish were divided and placed into two separate recycling (90%) systems. Each system (910 L) consisted of a head tank which contained biofilters and six tanks (125 L). Water quality was maintained as previously described. Sixteen fish from each of the six experimental groups were chosen at random at 12 wk, sacrificed and weighed. The feed rate was 0.52% of fish body weight per day for the 12 wk

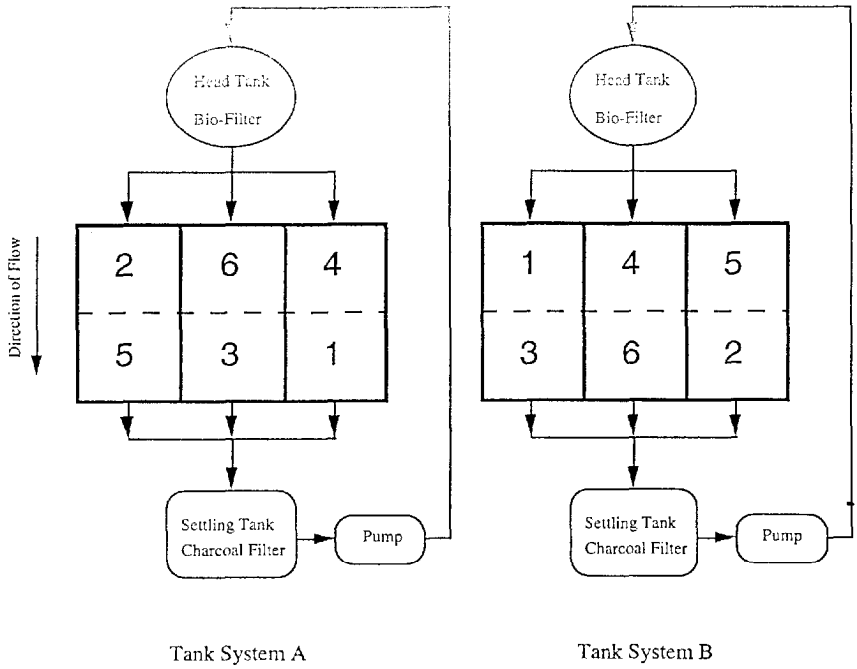

Figure 1. Diagram of tank systems. Numbers indicate dieldrin levels in feed which were given to fish in that tank (1=0; 2=0.01 ppm; 3=0.10 ppm; 4=1.0 ppm; 5=10.0 ppm; 6=25.0 ppm).

period. Estimated total feed for the fish collected at 12 wk was 34.3 g feed per fish.

For the first study, eight fish from each tank were collected at the 6 and 12 wk sampling intervals and four fish were combined as a subsample. For the second study, eight fish from each tank were collected at the 12 wk sampling interval and four of these were combined as a subsample. Subsamples of fish were ground 2 times through a meat grinder (Model 31FG10, Waring Products Div., New Hartfort, CN), placed in a glass jar, and stored at -20°C.

Extraction of fat and pesticide from the fish and feed was performed by a Soxhlet extraction. Samples were removed from the freezer and allowed to thaw for 20 min in warm water. Samples (5.0 g) were weighed into a 100 mL beaker and mixed with 55.0 g of anhydrous sodium sulfate (Baker Analyzed, ACS Reagent Grade, J.T. Baker, Phillipsburg, NJ). Samples were then placed in a desiccator at room temperature and held for 12 hr. After drying, the mixture was transferred into a 33 x 80 mm single thickness cellulose thimble (Whatman International Ltd., Maidstone, England) and covered with pre-rinsed glass wool (Pyrex®, Corning, Inc., Corning, NY). Samples were then extracted with a Soxhlet apparatus for 7 hr with 250 mL of hexane (Ultra-Resi Analyzed, J. T. Baker) at a turnover rate of

4 to 5 times per hr. The extract was concentrated to under 10 mL using a Kuderna-Danish concentrator (Model 120 S-Evap, Organomation, Inc., S. Berlin, MA) set at 90°C. The extract was transferred to a pre-weighed, pre-marked 50 mL screw cap centrifuge tube. Under a continuous flow of nitrogen, the remaining solvent was evaporated (Model 112 N-EVAP, Organomation, Inc.) while samples were held at room temperature. To determine the fat content, the extract was held for 2 hr in a fume hood and then weighed.

Liquid-liquid extraction was used to separate the pesticide from the fat. The volume of the sample was adjusted to 15 mL with petroleum ether (Ultra-Resi Analyzed, J.T. Baker). The extract was partitioned 3 times by shaking vigorously for 2 min with 30 mL of acetonitrile (Ultra-Resi Analyzed, J.T. Baker) saturated with petroleum ether. Each time, the acetonitrile layer was removed and placed into a 1 L separator funnel which contained 650 mL of deionized (DI) water, 40 mL of saturated NaCl (GR Crystals, EM Science, Gibbstown, NJ) solution and 100 mL of petroleum ether. This mixture was shaken for 1 min and the aqueous layer was drained into a second 1 L separatory funnel. Petroleum ether (100 mL) was added to the second funnel and shaken for 1 min. The aqueous layer was removed and the petroleum ether layers were combined and rinsed twice using 100 mL portions of DI water mixed with 5 mL of saturated sodium chloride solution. The remaining petroleum ether was filtered through anhydrous sodium sulfate and concentrated to 5 mL. The concentrate was transferred into a 22 mm i.d. chromatographic column containing 20 g of cooled, activated florisil (F-100, 30-60 mesh, Fisher Scientific, Fairlawn, NJ), topped with 1 cm of sodium sulfate. The florisil was activated by placing 10 g in 16 x 150 mm culture tubes and holding for more than 24 hr at 130°C. The column was pre-rinsed with 50 mL of petroleum ether and diethyl ether (Ultra-Resi Analyzed, J. T. Baker). The first solution contained 6% diethyl ether and 94% petroleum ether, and the second contained 15% diethyl ether and 85% petroleum ether. Two fractions (200 mL) were collected and concentrated to a volume of less than 5 mL. Final volume of the second fraction was adjusted to 5 mL and an aliquot of the extract was transferred into 2 mL crimp cap injection vials for analysis.

Analysis was performed using a gas chromatography (Model HP-5890 Series II, Hewlett-Packard, Avondale, PA) equipped with an autosampler and an electron capture detector. The sample (1 µL) was separated using an HP-5, 30 m x 520 µm megabore column (Hewlett-Packard Co.). An isothermal temperature program was used with inlet, oven and detector temperatures of 230°C, 220°C, and 300°C, respectively. Helium (Grade 5.0, Selox-Airco, Atlanta, GA) was used as the carrier gas with a flow rate of 1.5 mL min^{-1} and nitrogen (Grade 5.0, Selox-Airco) was used as the makeup gas with a flow rate of 60.0 mL min^{-1}.

Statistical analyses were conducted on the data collected from randomized complete block design experiments using analysis of variance and protected-$LSD_{0.05}$ which was used to determine significant differences between treatment

means. Multiple regression analysis was conducted using commercial software (Statview 4.1, Abacus Concepts Inc.).

RESULTS AND DISCUSSION

There was some difficulty in getting the fish to consume the formulated diets at the start of each study. The average weight of striped bass was not significantly ($p > 0.05$) affected by tank system or by diet but was affected by duration of the studies. Fish had an average weight of 80.9 and 98.1 g at 6 and 12 wk of the first study, respectively. Average weight of fish sampled at 12 wk of the second study was 88.9 g. Therefore, dieldrin concentrations below 25 ppm in feed did not significantly affect weight gain of fish throughout the 12 wk studies. Fat content in striped bass was significantly affected by feed contaminant level and by the interaction between duration of feeding and tank system, in the first study. The average fat content in fish for 6 and 12 wk of the first study was 12.4 and 11.4%, respectively. Fat content was significantly ($p > 0.05$) affected by an interaction between feed contaminant level and tank system in the second study. The average fat content was 10.2% for fish fed 12 wk in the second study.

Results from feeding dieldrin contaminated diet to striped bass for 6 and 12 wk are presented in Figure 2. Dieldrin was detected in control fish because water was recirculated through the system. There were no significant differences in dieldrin concentrations between fish from the 2 tank systems for either study. Dieldrin concentrations ranged from 0.125 to 6.197 ppm (fresh weight), in the first study, and from 0.673 to 8.095 ppm, in the second study. Dieldrin concentrations ranged from 1.105 to 57.458 µg dieldrin g^{-1} fat for the first study and 7.028 to 72.550 µg dieldrin g^{-1} fat for the second study. As expected, there was a significant increase in dieldrin in fish as the concentration in feed increased. There were no significant differences in tissue dieldrin concentration at 6 and 12 wk when fish were fed 0, 0.01, and 0.10 ppm diets. However, the dieldrin concentration in fish sampled at 12 wk was significantly higher than those sampled at 6 wk when fish were fed the 1.0, 10.0, and 25.0 ppm diets. Dieldrin concentrations at 12 wk were greater in fish from the second study because these fish came onto the diets more readily and hence consumed more of the dieldrin contaminated feed.

Multiple regression analysis indicates a relationship between dieldrin concentration and duration of feeding, concentration of dieldrin in diet, average weight of the fish, and total fat in the fish. The regression equation ($Y' = 0.2X_1 + 0.2X_2 + 0.0047X_3 - 0.1X_4 - 1.4$) describes this relationship ($R^2=0.90$). Where Y' = dieldrin concentration in fish (µg dieldrin/g fresh weight); X_1 = duration of feeding (wk); X_2 = concentration of dieldrin in diet (ppm); X_3 = average weight of fish (g); and X_4 = total fat in fish (g)

For future feeding studies, it would be possible to predict the concentration of dieldrin in striped bass if the duration of feeding, the concentration of dieldrin

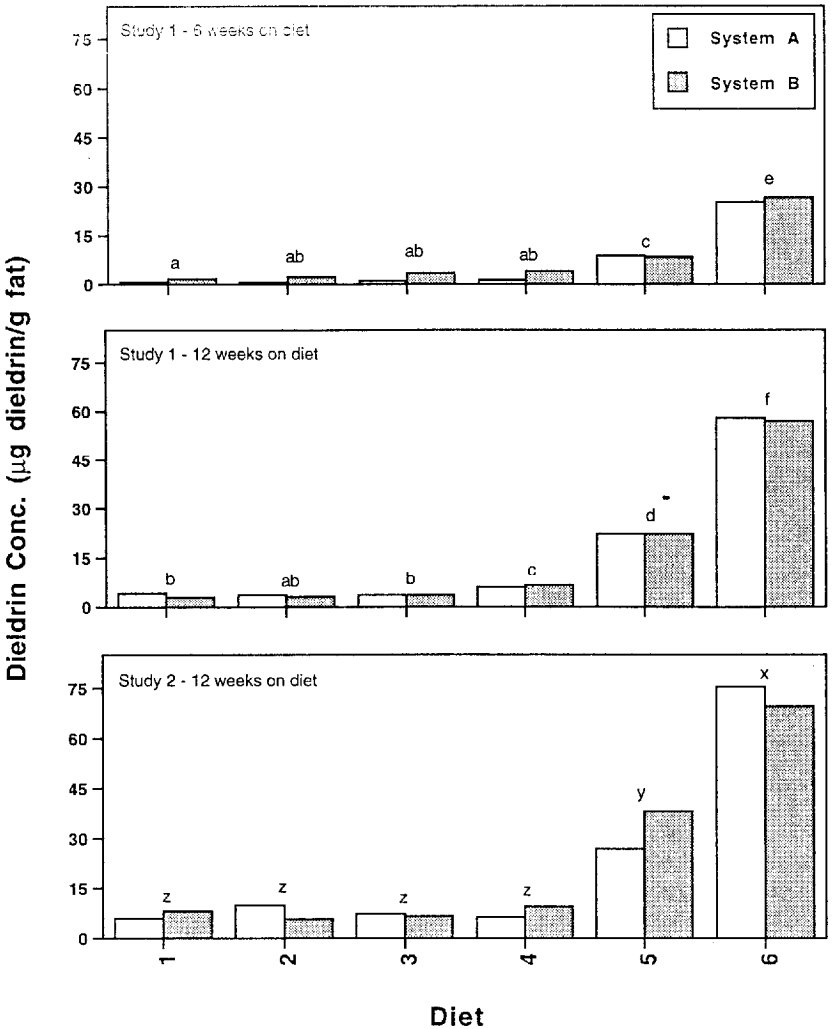

Figure 2. Dieldrin concentration based upon fat content of fish. Bars with different letters are significantly different at the p=0.05 level. Diet numbers indicate dieldrin levels in feed (1=0; 2=0.01 ppm; 3=0.10 ppm; 4=1.0 ppm; 5=10.0 ppm; 6=25.0 ppm).

received in the diet, the average weight of the fish and the total fat are known.

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