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Accumulation of Dietary DDE and Dieldrin by Largemouth Bass, *Micropterus salmoides floridanus*

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Long-term administration of toxicants to fish in an experimental setting presents a number of challenges. Not only must the toxicant be delivered consistently and reliably in an ecologically relevant fashion, stress due to captivity or repeated handling during dose administration and sample collection must be minimized to avoid compromising the accurate measurement of various toxicity end-points. Invasive administration techniques have been used in an attempt to maximize reliability and reproducibility of dosing, but these lack ecological relevance and introduce unwanted stress. Thus, a better method of experimental dosing is needed to improve fish studies.

In wild fish populations, the food web is the primary route of exposure to persistent organochlorine pesticides (OCPs) (Woodwell et al. 1967). Varying degrees of accumulation have been reported following experimental dietary exposure to DDT or dieldrin. In those studies, feed pellets were coated with an oil carrier that contained pesticide and the pellets were fed to fish at rates of 0.5–4.0% of body weight per day. Rainbow trout (*Oncorhynchus mykiss*) accumulated 20-24% of the available DDT dose over 140 days (Macek et al. 1970); brook trout (*Salvelinus fontinalis*) accumulated 35% over 120 days (Macek and Korn 1970); and Atlantic menhaden (*Brevoortia tyrannus*) accumulated 17-27% over 48 days (Warlen et al. 1977). Dietary dieldrin accumulation was approximately 10% for both rainbow trout over 140 days (Macek et al. 1970) and striped bass (*Morone saxatilis*) over 84 days (Santerre et al. 1997).

The largemouth bass (*Micropterus salmiodes*) is a species of ecological importance in which high contaminant concentrations, depressed sex steroids, reversed sex steroid ratios, and reduced survival of fry have been observed in contaminated areas of Florida (Benton and Douglas 1996; Marburger et al. 1999). Dietary accumulation studies have not been reported for OCPs in largemouth bass. Here we report the results of a pilot study that compared the effectiveness of sinking versus floating feeds as dietary administration routes for *p,p'*-DDE (DDE) and dieldrin in largemouth bass. We sought to determine the variability of DDE and dieldrin accumulation inherent to each method and to determine their effects on circulating sex steroid levels after 50 days of exposure at the onset of reproductive season.

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MATERIALS AND METHODS

Largemouth bass, 1.5 - 2 years of age were housed in groups of 14 fish in 700 L round tanks equipped with a flow-through system supplied by on-site well water and aeration (water quality parameters measured daily). On Day 0 of the experiment (December 5, 2002) bass had an average weight, length, and condition factor (K) (± SD) of 181.6 ± 34.2 g, 233.2 ± 13.3 mm, and 1.42 ± 0.10, respectively, indicating fish were healthy and of reproductive size.

p,p'-DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethylene, Lot # 09020KU, 99.4% purity) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene, Lot # 077H3578, 91.2% purity) were obtained from Aldrich Chemical Company (Milwaukee, WI, USA) and shipped to Zeigler Brothers, Inc. (Gardners, PA, USA) where they were incorporated into sinking or floating feed pellets containing no chemical (control), 5 ppm DDE, or 1 ppm dieldrin. Sinking pellets were ground into powder then mixed with pesticide and reconstituted; floating pellets were top-dressed with fish oil mixture containing pesticide. Background contaminant analysis indicated low levels of aldrin (0.03 ppm) in all feed. Treated or control feed (2 replicates each) was administered to each tank at 5% mean total tank body weight per day (recommended by Rick Stout, FFWCC-Fish Hatchery, Richloam, FL, USA). During the study, fish ate only 1% body weight per day and so the feeding rate was adjusted. Pellets not consumed were counted daily and the weight of consumed pellets calculated.

On Days 30 (n = 3) and 50 (n = 11), weight and length were measured and K was calculated according to the following formula: K = weight in grams/length in millimeters^3 x 100,000. Blood was collected and plasma separated by centrifugation, fish were sacrificed, and gonads were removed and weighed. Each fish carcass (and separated gonad for Day 50 fish) was wrapped in aluminum foil, placed in a plastic bag and labeled. Carcasses and gonads were analyzed by gas chromatography – mass spectrometry (GC-MS) for DDE and dieldrin. GC-MS analysis, following EPA method 8270 (US EPA 1990), was conducted by the Center for Environmental and Human Toxicology, University of Florida. Recovery ranged between 65 and 100% (detection limit of 0.75-1.5 ng/g).

Blood plasma from largemouth bass was analyzed in duplicate for 11-ketotestosterone (11-KT) and 17β-estradiol (E_2) using a previously validated ^{3}H radioimmunoassay (RIA) method (Gross et al. 2002) and values reported as pg of hormone / ml of plasma. Standard curves were prepared in phosphate buffered saline plus gelatin and sodium azide (PBSGA) with known amounts (15, 30, 60, 125, 250, 500, 1000, and 2000 pg) of radioinert E_2 (ICN Biomedicals, Costa Mesa, CA, USA) or 11-KT (Sigma Chemicals, St. Louis, MO, USA) and 15,000 cpm of ^{3}H-E_2 or ^{3}H-11-KT. Antibodies were purchased from ICN Biomedicals (E_2) or Helix Biotech, Richmond, BC, Canada (11-KT). Coefficients of variation (CV%) for the standard curves (E_2 and 11-KT) were less than 6.5%. Sample CV% was less than 15% or the samples were rerun. The minimum concentrations

distinguishable from zero for all assays were (mean ± SE) 148 ± 18 pg/ml for E_2 and 29 ± 18 pg/ml for 11-KT. Cross-reactivities (characterized by T.S. Gross, University of Florida) of the E_2 antiserum with other steroids were: 11.2% for estrone, 1.7% for estriol, and < 1.0% for 17β-estradiol and androstenedione. Cross reactivity of the 11-KT antiserum with other steroids was: 9.7% for testosterone, 3.7% for α-dihydrotestosterone, and < 1.0% for androstenedione.

All parameters were analyzed using the Statistical Analysis System (SAS), version 9. The Means Procedure was used to determine averages and standard deviations for all treatment groups and replicates, and the data presented are means ± SD. An analysis of variance (ANOVA) procedure followed by the Duncan's Multiple Range Test was used to test differences between replicates, feed type, and chemicals. Significance was declared at $\alpha = 0.05$.

RESULTS AND DISCUSSION

There was no mortality throughout the study. While biological loading was relatively high, flow rates insured rapid water exchange and water quality remained in healthy ranges. K at Days 30 and 50 were 1.45 ± 0.09 and 1.42 ± 0.10, respectively, and did not differ between feed types or pesticide administered, indicating that a healthy population was maintained. Regardless of sex, largemouth bass grew an average of 3 ± 0.4 mm and gained an average of 2 ± 0.8 g over the course of the study.

After 30 days of exposure, treated bass had accumulated significant levels of DDE or dieldrin, regardless of feed type (Figure 1). Fish fed sinking DDE pellets consumed approximately 200 µg of DDE per animal. The whole-body DDE concentrations differed between replicates for sinking DDE feed (Figure 1A), but the percentage of the total dose accumulated did not differ between replicates due to slightly higher consumption in one replicate (Figure 2A). Fish fed floating DDE pellets consumed approximately 190 µg of DDE per fish and there were no differences between replicates with respect to whole-body DDE concentrations or percentages of the total dose accumulated (Figures 1A and 2A). Fish fed sinking dieldrin pellets consumed approximately 40 µg per fish over 30 days and whole-body dieldrin concentrations and percentage of the total dose accumulated did not differ between replicates (Figures 1C and 2B). Fish given floating dieldrin feed consumed approximately 35 µg of dieldrin over 30 days. The whole-body dieldrin concentrations and percentages of the total dose accumulated did not differ between replicates for floating dieldrin feed (Figures 1C and 2B). As evidenced by smaller standard deviations from the mean, the floating DDE and dieldrin diets produced a more consistent dose than the sinking diets.

Floating pellets produced less variability in dose at Day 30 among fish within a tank, as well as among replicates. Because it produced more consistent dosing, floating feed was selected for further method validation and determination of effects on circulating sex steroids. DDE and dieldrin concentrations were

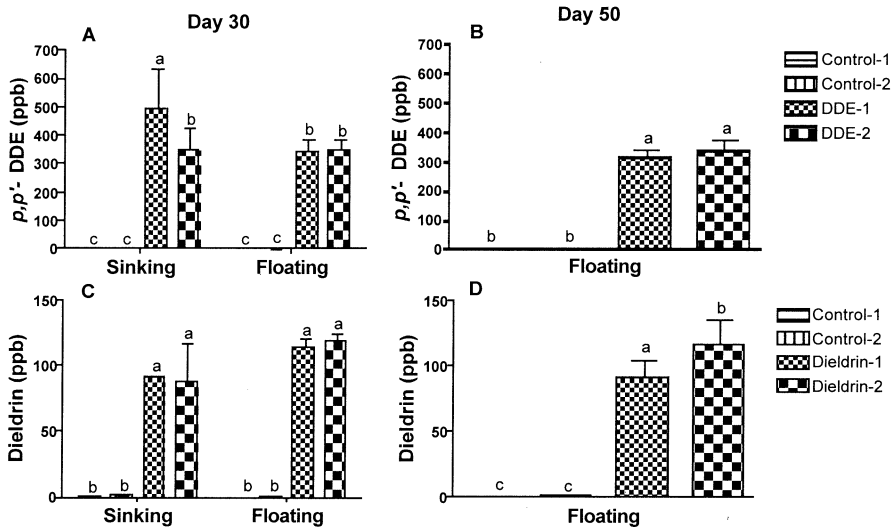

Figure 1. Whole-body concentration ± SD of DDE (A,B) and dieldrin (C,D) at Days 30 and 50 (n = 3 per treatment). Letter designations indicate a statistically significant difference. Numbers in legend indicate replicates.

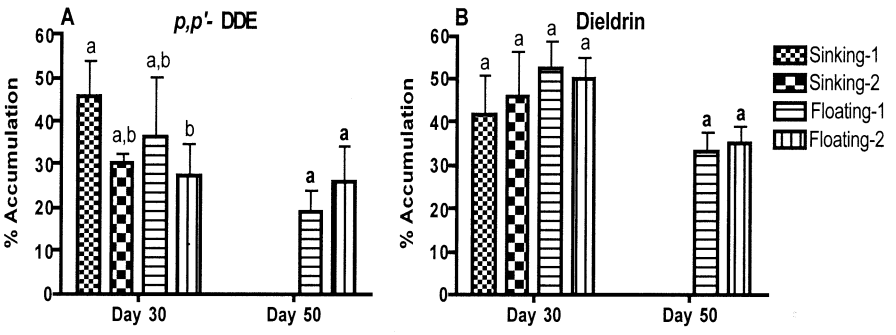

Figure 2. Percentage of total DDE (A) or dieldrin (B) dose accumulated ± SD within each replicate (n = 3 per replicate). Letter designations indicate a statistically significant difference. Numbers in legend indicate replicates.

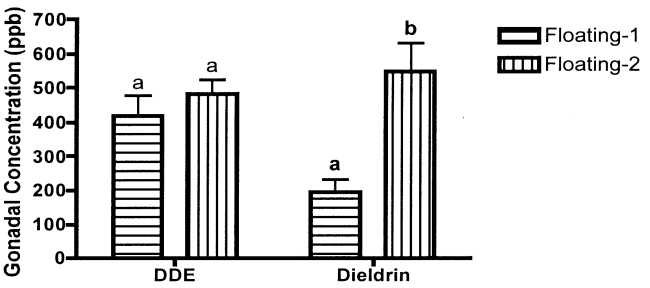

Figure 3. Day 50 gonadal concentrations of DDE and dieldrin (n = 3, male and female). Letter designations indicate a statistically significant difference. Numbers in legend indicate replicates.

determined at Day 50 for a subset of three fish from each replicate fed floating diets. Background contaminant concentrations were assumed to remain unchanged from Day 30, so contaminants were not analyzed in day 50 controls. Fish administered floating DDE consumed approximately 275 µg DDE over 50 days. The whole-body DDE concentrations and percentage accumulated did not differ between replicates (Figures 1B and 2A), nor did gonadal DDE concentrations (Figure 3). The gonad dose contributed approximately 0.5% and 1.0% of the whole-body dose for males and females, respectively. Fish in tanks administered floating dieldrin feed consumed different amounts over 50 days. Replicate 1 consumed less total feed and each fish consumed approximately 47 µg dieldrin, while the second replicate consumed approximately 57 µg dieldrin per fish, a 20% difference. Whole-body dieldrin concentrations were significantly different between replicates, which is consistent with the difference in dieldrin consumption (Figure 1D). However, the percentage of the total dose accumulated did not differ between replicates (Figure 2B). Gonadal dieldrin (Figure 3) contributed approximately 1.0% (Replicate 1) and 3.5% (Replicate 2) to the whole-body concentrations, with no apparent difference between males and females. Gonad concentrations of both OCPs were variable between replicates, but were generally consistent with respect to the percentage of whole-body concentration. DDE showed a greater partitioning into the female gonad than the male, most likely due to the high lipid content of developing oocytes within the female gonad. Dieldrin did not show this difference in partitioning between the sexes, but a difference between replicates makes interpretation difficult. It is possible that the percentage stored in the fatty tissue of the gonad increases with the dieldrin dose administered. A larger sample size might help to determine whether gonadal OCP partitioning truly differs between sexes.

The results of this study suggest that diets containing OCPs provide an accurate and consistent route of administration for largemouth bass with only moderate variability within replicates. Sinking style feed produced greater variability both within and between replicates. This variability was most likely due to competition among fish for the pellets as they sank because bass are usually top

feeders and will not eat pellets that reach the bottom of the tank. The floating style feed was more natural for bass and created greater dose consistency. In addition, the pesticide concentrations within floating feed pellets were less variable and closer to the target doses than the sinking feed. The consistency achieved with the floating feed, within both the pellets and the fish, suggests superiority over sinking feed as an exposure route for largemouth bass.

The rapid uptake of both DDE and dieldrin from the diet of largemouth bass is consistent with the results of Grzenda et al. (1971) that demonstrated rapid uptake for both pesticides in goldfish (*Carassius auratus*) fed contaminated diets. Although both the Grzenda et al. study and this study continued exposure past the initial uptake phase, the accumulation rate leveled off after 30 days of exposure and tissues maintained a consistent dose, indicating a steady state was attained (Figure 1). In Atlantic menhaden, equilibrium was not reached during 60 days of oral DDT exposure (Warlen et al. 1977). In rainbow trout, 140 days of dietary exposure was required to reach equilibrium for DDT and dieldrin (Macek et al. 1970). In our study, the amounts of DDE and dieldrin accumulated comprised 30% and 50% of the total dose, respectively, at Day 30, and 10% and 15%, respectively, at Day 50. This could be due to tissue saturation or metabolic factors such as a decrease in absorption efficiency or an increase in biotransformation and elimination of the absorbed dose. Elevated elimination rates most likely account for the decrease in overall accumulation because OCPs are known to induce liver biotransformation enzymes at high doses in fish (Zapata-Pérez et al. 2000; Foster et al. 2001).

Our results in bass are consistent with other studies on DDE/DDT accumulation but inconsistent with studies on dieldrin accumulation in various fish species. For DDT, uptake was between 20 - 35% in several studies on rainbow trout, brook trout and Atlantic menhaden over periods of 140, 120, and 48 days, respectively (Macek and Korn 1970; Macek et al. 1970; Warlen et al. 1977). Here bass accumulated a total of 20 - 25% of the DDE dose at Day 50. For dieldrin, rainbow trout (Macek et al. 1970) and striped bass (Santerre et al. 1997) had only a 10% accumulation over 140 and 84 days, respectively, while the largemouth bass in this study accumulated 30 - 35% of the total dieldrin dose after 50 days. The higher accumulation and/or retention of OCPs and other lipophilic compounds may be due to the high lipid content of largemouth bass, which is between 6 and 10% and which increases during the spawning season due to the development of the gonad (Barziza and Gatlin 2000). In addition, the largemouth bass in this study achieved environmentally relevant doses of both chemicals by consumption of pesticide-treated feed. Whole-body concentrations of treated fish were approximately 375 ppb for DDE and 100 ppb for dieldrin. The average gonadal pesticide concentrations achieved in this study were 6-fold lower and 2-fold higher than the DDE and dieldrin concentrations reported in bass from contaminated areas of the Ocklawaha River basin in Central Florida (Marburger et al. 1999). The wild fish found in these areas also have depressed sex steroid profiles.

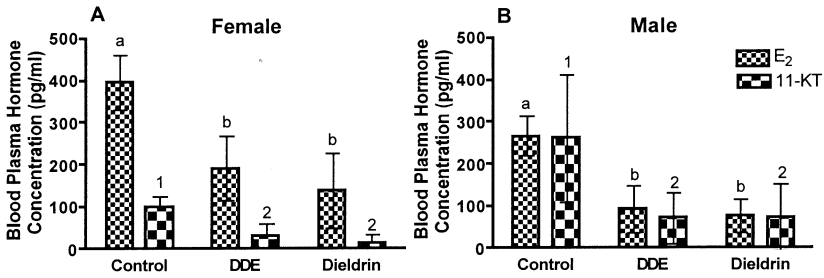

Figure 4. Day 50, circulating hormones ± SD (female *n* ranged from 7 to 8; male *n* ranged from 14 to 15). Different letter (E_2) / number (11-KT) designations over bars indicate a statistically significant difference.

Both DDE and dieldrin produced effects on the endocrine systems of treated fish at the concentrations achieved in this study. Although GSIs for DDE and dieldrin treated fish (males and females) were not significantly different from controls and did not increase over time, as would be expected at this time of year, sex steroid concentrations (E_2 and 11-KT) in treated males and females were significantly lower than controls and did not differ between replicates (Figure 4). Additionally, although the whole body dieldrin concentration differed between replicates at Day 50, the magnitude of the decrease in sex steroid hormones did not differ, indicating a difference of approximately 20 ppb may not be biologically relevant regardless of statistically significant differences in dose. The GSI and circulating level of sex steroids from Day 50 control fish were 5 to 10 times and 10-fold lower, respectively, than what has been shown for both male and female adult hatchery-raised bass (Gross et al. 2002), indicating that the fish used in this study were not sexually mature. It is possible that depressed seasonal sexual maturation may have been due to stress induced by captive conditions.

In conclusion, exposing largemouth bass to OCPs by incorporation into an oil carrier and subsequent coating of feed pellets is an effective, accurate, and reproducible dosing method. Variability in whole-body concentrations achieved and percentage of total dose accumulated was minimal for treatment with either DDE or dieldrin. A rapid uptake followed by a sustained whole-body concentration after 30 days indicates that study length should be a minimum of one month. In addition, effects of these OCPs on endocrine function during the onset of reproductive season were clearly manifest after 50 days of continuous exposure, with treated fish exhibiting depressed sex steroid profiles. To optimize the oral exposure method for largemouth bass, further research should be conducted to examine OCP accumulation (absorption and elimination) over a longer period of time and during different seasons throughout the year.

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