

INFLUENCE OF SEASONALITY AND EXPOSURE ON THE ACCUMULATION AND REPRODUCTIVE EFFECTS OF *p,p'*-DICHLORODIPHENYLDICHLOROETHANE AND DIELDRIN IN LARGEMOUTH BASSKEVIN G. JOHNSON,<sup>†</sup> JENNIFER K. MULLER,<sup>‡</sup> BERTRAM PRICE,<sup>§</sup> ADAM WARE,<sup>§</sup> MARÍA S. SEPÚLVEDA,<sup>||</sup> CHRISTOPHER J. BORGERT,<sup>‡#</sup> and TIMOTHY S. GROSS\*<sup>#</sup><sup>†</sup>Department of Fisheries and Aquatic Sciences, University of Florida College of Agricultural and Life Sciences, Gainesville, Florida 32653, USA<sup>‡</sup>Applied Pharmacology and Toxicology Inc., Gainesville, Florida 32605, USA<sup>§</sup>Price Associates, White Plains, New York 10601, USA<sup>||</sup>Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907, USA<sup>#</sup>Center for Environmental and Human Toxicology, Department of Physiological Sciences, University of Florida College of Veterinary Medicine, Gainesville, Florida 32611, USA

(Received 5 July 2006; Accepted 2 November 2006)

**Abstract**—Two studies investigated the accumulation and reproductive effects of *p,p'*-dichlorodiphenyldichloroethane (DDE) and dieldrin over 30 or 120 d of oral exposure in captive Florida, USA, largemouth bass (*Micropterus salmoides floridanus*). The 30-d exposures were conducted during the peak reproductive season, and the 120-d study was conducted to simulate exposure throughout the ovarian cycle. Whole body chemical residue concentrations were similar, regardless of exposure duration, for the medium and high feed concentrations of either chemical; however, the low-dose residue concentrations were much lower, yet similar to natural exposures. No clear dose-response relationships were identified between chemical dose and morphological (length, weight, hepatosomatic index) or reproductive endpoints (sex steroid concentration, gonadosomatic index, percentage of fry hatching). Reproductive parameters were variable within treatment groups, indicating that circulating sex steroids and percent hatch endpoints have high natural variability among fish of the same age and reproductive stage. However, in general there was a decrease in plasma estradiol and 11-ketotestosterone for female and male fish, respectively, that were exposed to dieldrin. Overall, results suggest that exposure throughout ovarian (follicular) development to either DDE or dieldrin alone does not result in the depressed endocrine status and poor reproductive success reported in highly organochlorine pesticide-contaminated environments in Central Florida, USA.

**Keywords**—Fish Organochlorine pesticide Dichlorodiphenyldichloroethane Dieldrin Reproduction

## INTRODUCTION

Controlled laboratory exposures and field studies have indicated that persistent organochlorine pesticides (OCPs) can affect steroidogenesis in fish [1–3]. Two of the most prominent OCPs found in the historically contaminated soils of central Florida's St. John's River Water Management District's Emerald Marsh Conservation Area (EMCA) are dieldrin and derivatives of DDT [4]. These chemicals are known to bioaccumulate in Florida largemouth bass (*Micropterus salmoides floridanus*) found in contaminated sites. Organochlorine pesticide concentrations found in largemouth bass ovaries and fat have been detected at concentrations greater than 4,000 ppb and 17,000 ppb, respectively, for total DDT derivatives and 100 and 700 ppb, respectively, for dieldrin [4,5]. In addition to high contaminant concentrations, depressed sex steroids (17 $\beta$ -estradiol and 11-ketotestosterone) and reversed sex steroid ratios have been observed in fish from this site [6,7]. Both male and female largemouth bass, captured from the EMCA and tagged for repeat analysis, had sex steroid concentrations that remained low throughout the year and showed no seasonal trends [7]. Stocked adult largemouth bass in the EMCA appeared to have limited reproductive success or recruitment to the fingerling stage [8].

The focus of the current studies was to investigate the effects of *p,p'*-dichlorodiphenyldichloroethane (DDE) and dieldrin on morphological and reproductive parameters in largemouth bass after exposure durations of 30 and 120 d during different portions of the seasonal reproductive cycle. Morphological and reproductive endpoints were measured either after 30 d of exposure at or near the seasonal spawning period in bass, or after 120 d of exposure occurring throughout the reproductive cycle. Pesticide doses were chosen to create a range of whole-carcass concentrations encompassing those reported for wild largemouth bass in reclaimed agriculture areas along the Ocklawaha River Basin (FL, USA) such as the ECMA. Target carcass concentrations were calculated assuming that only 30% of the DDE and 50% of the dieldrin ingested would accumulate in body tissues [3]. Characterization of the effects of these two pesticides may assist in determining if they act alone, or as part of a more complex mixture, in impairing the reproductive success of largemouth bass found in waterways contaminated with OCPs. Additionally, comparison of morphological and endocrine responses after relatively short or long exposure durations should provide insight into the dosing conditions and timing needed to generate the types of effects seen in wild fish. Our objectives were to determine the dose of DDE and dieldrin accumulated after 30 and 120 d of oral exposure (exposure duration prior to peak spawning season); the dose-response relationship between sublethal doses

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of DDE and dieldrin and several health and reproductive endpoints in largemouth bass; and the effect of exposure duration on these relationships.

## MATERIALS AND METHODS

### *Experimental animals*

Hatchery-reared, two-year-old Florida largemouth bass were obtained from American Sports Fish (Montgomery, AL, USA) in March (30-d exposure) and October (120-d exposure) 2003. Fish were maintained at the U.S. Geological Survey Biological Resource Division Center for Aquatic Resource Studies (USGS-BRD-CARS) facility in Gainesville (FL, USA) in groups of 20 fish (10 males and 10 females) in 18 separate 700-L round tanks for the 30-d exposure, or in groups of 100 fish in seven separate 6,000-L concrete raceways for the 120-d exposure, both equipped with a flow-through system supplied by on-site well water and aeration. Before experimentation began, largemouth bass had mean  $\pm$  standard deviation weights of  $150 \pm 34$  g and  $160 \pm 36$  g, respectively.

Water quality parameters (temperature, dissolved oxygen, pH, and ammonia) were measured twice per week. All parameters were within acceptable ranges for the entire duration of both experiments: Temperature ( $20.6$  to  $22.7^\circ\text{C}$  and  $12.2$  to  $22.4^\circ\text{C}$  for the 30- and 120-d exposures, respectively), dissolved oxygen ( $6.74$  to  $8.60$  mg/L and  $7.06$  to  $11.75$  mg/L for the 30- and 120-d exposures, respectively), pH ( $7.6$  to  $8.0$  regardless of exposure duration), and ammonia content ( $<1$  mg/L regardless of exposure duration).

### *Experimental design*

**Thirty-day exposure duration.** Largemouth bass were fed chemically treated floating pellet feed daily for 30 d beginning March 21, 2003. The 30-d exposure utilized 440 largemouth bass (220 males and 220 female) placed randomly into nine pesticide treatments and two control treatments in duplicate, each tank containing 10 males and 10 females. After day 30, five to six fish of each sex were sacrificed per replicate to collect body tissues for contaminant analysis.

**One hundred and twenty-day exposure duration.** Largemouth bass were fed chemically treated floating pellet feed daily for 120 d beginning November 4, 2003. The 120-d exposure utilized 700 largemouth bass placed randomly into six treatments or control treatment in duplicate ( $n = 50/\text{treatment duplicate}$ ). Sex ratio of the 120-d treatments was unknown at the study initiation because gender cannot be determined noninvasively this early in the reproductive cycle (November). On day 0 of the 120-d exposure, an additional 24 fish were sacrificed for collection of baseline physiological data (organ weights and sex steroid hormone concentrations). Five to six fish of each sex were collected per replicate at days 30, 60, 90, and 120. Results were summarized for the 120-d exposure in this report because significant exposure trends were neither detected nor consistent.

Fish for each exposure were collected and processed to examine morphological and reproductive endpoints. Additionally, five female largemouth bass were collected from the Emerald Marsh Conservation Area 7 for whole-carcass and gonad analysis to determine if ecologically relevant chemical/contaminant concentrations were attained in the laboratory.

### *Chemicals and feed*

The 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (*[p,p'*-DDE], lot 09020KU 99% purity [30-d], A016284201 99%

purity, and A012306101 99% purity [120-d]) 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 77H3578, 90% purity) were obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA) and Acros Organics (Morris Plains, NJ, USA). The pesticides were mixed into menhaden fish oil to form two separate concentrated stock solutions at the USGS-BRD-CARS facility. The stock solutions then were shipped to Zeigler Brothers (Gardners, PA, USA) where they were incorporated into Ziegler's floating pellet fish feed (commercial diet). This diet routinely has been utilized for the maintenance of largemouth bass and without any negative effects on health and reproductive function [2,3,9]. The pesticide-laden floating feed was manufactured by first serially diluting the stock solution into additional menhaden fish oil to obtain the solutions needed to create the feed concentrations listed below. This fish oil/stock solution mixture then was added into a mixer containing the pellet feed and mixed thoroughly to achieve consistent coating of all pellets. Control feed was manufactured by combining the floating pellet feed with a top dressing of pure menhaden fish oil.

New Jersey Feed Laboratory (Trenton, NJ, USA) tested samples of each feed type and dose for chlorinated pesticides and polychlorinated biphenyls. Polychlorinated biphenyls were not detected in any feed type; OCPs were not detected in control feed; and DDE and dieldrin were only detected in the respective treated feed. For the 30-d exposure, target feed concentrations of 1, 7, 37, and 185 mg/kg DDE and 0.02, 0.1, 0.6, and 3 mg/kg dieldrin had actual concentrations of 1.06, 7.39, 34.5, and 136 mg/kg DDE, respectively, and 0.03, 0.1, 0.64, and 4.69 mg/kg dieldrin, respectively. For the 120-d exposure, target feed concentrations of 7, 37, and 185 mg/kg DDE and 0.1, 0.6, and 3 mg/kg dieldrin had actual concentrations of 5.3, 45.9, and 50 mg/kg DDE and 0.04, 0.4, and 0.81 mg/kg dieldrin, respectively. It is most likely that the feed manufacturer made significant errors in the dilution of pesticide stock solutions, which resulted in the low detected concentrations for the high doses of both DDE and dieldrin for the 120-d exposure study.

### *Feeding rate*

Feed was administered to each tank at 1% mean tank body weight per each tank as described by Muller et al. [3]. Feed doses of 1, 7, 37, and 185 mg/kg DDE were predicted to result in final carcass concentrations of 0.1, 0.63, 3, and 17 mg/kg DDE, respectively, based on 30% accumulation over 30 d. Feed doses of 0.02, 0.1, 0.6, and 3 mg/kg dieldrin were predicted to result in final carcass concentrations of 0.0018, 0.009, 0.054, and 0.27 mg/kg dieldrin, respectively, based on 50% accumulation. The amount of feed administered per tank over the 120-d exposure was adjusted every 30 d after removal of fish for sampling to maintain a similar amount of feed per fish throughout the experiment.

### *Endpoints*

Weight (g) and length (mm) were measured and blood was collected from all fish at the end of the 30-d exposure and for 12 fish (6 male and 6 female) per treatment every 30 d from the 120-d exposure. Exposure was ended and samples were collected from late March to mid-April for both studies (primary spawning season or period). Condition factor was calculated as  $K = \text{weight}/\text{length}^3 \times 100,000$ . Approximately 1 ml of blood was collected from the caudal vein of each fish

with a 3-ml syringe and heparinized 20-gauge 3.81-cm needle. Blood was dispensed into a 3-ml heparinized vacutainer, labeled, and stored on ice until centrifuged at  $1,000 \times g$  at  $4^\circ\text{C}$  for 15 min to separate red blood cells from plasma. Plasma was removed, placed into cryovials, and stored at  $-80^\circ\text{C}$  for later analysis of circulating sex steroids. After bleeding, each fish was euthanized with a blow to the head and sex was determined by examining gonadal morphology. The liver and gonads were excised from all fish and weighed to the nearest 0.01 g for determination of hepatosomatic index (= liver weight/fish weight  $\times 100$ ) and gonadosomatic index (= gonad weight/fish weight  $\times 100$ ). A cross-section of each gonad also was collected, placed in a histological cassette, and fixed in 10% buffered formalin for later histological analysis. The remaining gonad tissue and carcass then were wrapped in aluminum foil, placed in a labeled plastic bag, and stored at  $-80^\circ\text{C}$  until contaminant analysis by gas-chromatography mass-spectroscopy. Contaminants were analyzed from carcass and gonad samples of one male and one female from each replicate of the 30-d exposure (total  $n = 2$  males and 2 females treatment). The whole carcass was analyzed for three males and three females from each treatment for the 120-d exposure. Final OCP concentrations from each treatment were pooled for graphical representation.

After the final sample collection of the 120-d exposure, a total of eight pairs of fish from each treatment and control were placed into separate 0.10-acre experimental ponds lined with spawning mat. Ponds were monitored daily and the first six clutches in each pond were collected. Nests (fertilized eggs) were removed by cutting out that portion of the mat, folding it over, gently raising it to the surface, and then placing it in a cooler filled with pond water for transport. In the lab, mat sections were removed from the cooler and placed in a 1.5% sodium sulfite solution for five to seven minutes to loosen the eggs from the spawning mat as described by Sepúlveda et al. [10]. Three sets of 100 live embryos from each clutch were separated and placed into McDonald hatching jars to hatch. Jars were monitored daily for embryo mortality or hatching, and each jar was treated daily with a static bath of hydrogen peroxide (500 ppm of 35% active ingredient) for 30 min to prevent fungal growth. Once hatching of the embryos in each jar was complete, the number of live fry was counted to characterize differences in hatch rate across treatments.

#### Gonad histology

Gonadal tissue samples were fixed in 10% buffered formalin, sectioned, mounted on slides, and stained with Mayer's hematoxylin and eosin by Histology Tech Services (Gainesville, FL, USA). Slides were analyzed under a light microscope at  $\times 40$  and stages of sexual maturation were assigned according to Gross et al. [9].

#### Analysis of circulating sex steroid hormones

Plasma samples of largemouth bass were analyzed for  $17\beta$ -estradiol ( $E_2$ ) and 11-ketotestosterone (11-KT) using a validated  $^3\text{H}$  radioimmunoassay procedure [9]. All plasma samples were assayed in duplicate and values were reported as pg/ml of plasma. Samples (50  $\mu\text{l}$ ) were extracted twice with diethyl ether prior to radioimmunoassay analysis and results were corrected for extraction efficiency (87 and 79% for estradiol and 11-KT, respectively). Standard curves (1, 5, 10, 25, 50, 100, 250, 500, and 1,000 pg) were prepared in phosphate-buffered saline plus gelatin and sodium azide with known amounts of

radioisotopes  $E_2$  (ICN Biomedicals, Costa Mesa, CA, USA) or 11-KT (Sigma Chemicals) and approximately 30,000 cpm of  $^3\text{H}$ - $E_2$  or  $^3\text{H}$ -11-KT (specific activities of 120–180 Ci/mmol). Antibodies against sex steroid hormones and phosphate-buffered saline plus gelatin and sodium azide buffer then were added to the sample tube and incubated overnight at  $4^\circ\text{C}$ . Antibodies were purchased from ICN Biomedicals ( $E_2$ ) or Helix Biotech, Richmond, British Columbia, Canada (11-KT). After incubation, unbound antibody was removed by the addition of dextran-coated charcoal and then centrifuged for 10 min at  $1,000 \times g$ . The supernatant was removed (400  $\mu\text{l}$ ) and added to a scintillation vial with 4 ml of Scintiverse scintillation cocktail (Fisher Scientific, Pittsburg, PA, USA). Samples vials then were placed into a liquid scintillation counter (Pachard Tricarb, Model 1600) and counted for two min. Radioimmunoassay results were analyzed using a 4-parameter logistics analysis (Beckman Immunofit software; Beckman Coulter, Fullerton, CA, USA). The minimum concentration distinguishable from zero (mean  $\pm$  standard deviation) was  $67 \pm 15$  pg/ml for  $E_2$  and  $46 \pm 19$  pg/ml for 11-KT for the 30-d exposure and  $89 \pm 33$  pg/ml for  $E_2$  and  $72 \pm 18$  pg/ml for 11-KT for the 120-d exposure. Crossreactivities of the  $E_2$  and 11-KT antiserum were characterized previously [9].

#### Analysis of largemouth bass tissues for OCPs

Organochlorine pesticide analysis was performed at the Center for Environmental and Human Toxicology, University of Florida (Gainesville, FL, USA). First, the carcass/gonad tissue was homogenized to eliminate any variability within the sample. A 2- to 5-g portion of each sample was extracted into ethyl acetate. The sample then was purified using C18 and NH<sub>2</sub> solid phase extraction cartridges. Total OCP content was determined using gas-chromatography mass-spectroscopy, according to methods described by Rauschenberger et al. [11]. Readings were not lipid normalized. Samples were analyzed multiple times in full scan mode for analyte identification and in selected ion mode for quantitation to improve sensitivity. Percent recovery for *p,p'*-DDE ranged from 90 to 98% with a method detection limit of 0.11 to 1.5 ng/g. Percent recovery for dieldrin ranged from 70 to 89% with a method detection limit of 0.46 to 1.5 ng/g.

#### Statistical analysis

Endpoints were analyzed with Statistical Analysis System (SAS Institute, Cary, NC, USA), Version 9 and dose-response curves estimated using the Hill model. Data did not meet parametric assumptions; therefore all analyses were conducted following log transformation, although results are presented as nontransformed means. Analyses of variance were performed and significance was declared at a *p*-value equal to or lower than 0.05. Duncan's multiple range test followed as a multiple comparison procedure to determine which treatments differed. The Hill model, which is displayed below, has three parameters and is sufficiently flexible to capture a broad range of dose-response patterns [12].

$$E[R] = \frac{(E_{\text{CON}} - B) \cdot \left(\frac{\text{DOSE}}{C}\right)^A}{1 + \left(\frac{\text{DOSE}}{C}\right)^A} + B$$

where  $E(R)$  is the expected value of the hormone measurement,  $A$  is the slope parameter,  $B$  is the background response (i.e.,

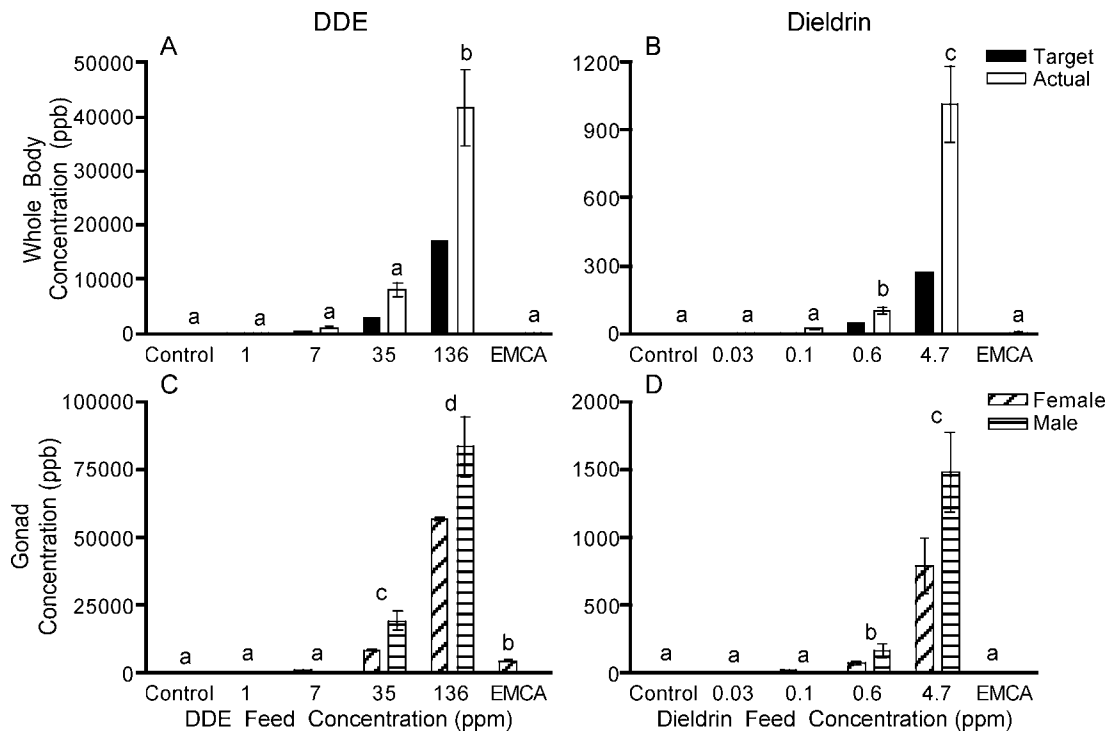


Fig. 1. Mean ( $\pm$  standard error) whole body concentrations for males and females combined (A, B) and mean female/male gonadal concentrations (C, D) of dichlorodiphenyldichloroethane (DDE) and dieldrin in fish exposed orally for 30 d ( $n = 2$  per treatment) and fish collected from Emerald Marsh Conservation Area 7 (EMCA) ( $n = 5$ ). Treatments with the same lowercase letter were not significantly different ( $p > 0.05$ ).

response due to the assay alone, which may be measured by a media blank), C is the median effective dose, DOSE is the applied dose, and ECON is the control response (i.e., amount of hormone in plasma when dose is zero). Our analysis included characterizations of dose-response curves (using the Hill model and linear regression analyses) where the data supported a statistical fit and we attempted to determine why there was no dose-response relationship where the statistical fit failed.

## RESULTS

### Largemouth bass

During these experiments, there was no mortality of largemouth bass in either study. Morphological indicators (weight, length, K, and hepatosomatic index), did not differ significantly between replicates, and pooled replicate values for

males and females did not differ between treatments. Tissue concentrations of DDE and dieldrin in the gonads and whole carcass were higher than targeted, but increased proportionally to feed concentrations after 30 d of exposure (Fig. 1). After exposure for 120 d, final tissue concentrations were within target range for the low dose of DDE and the low and high doses of dieldrin (Fig. 2). However, tissue concentrations from high-dose fish in the 120-d exposure were much lower than fish administered the high dose for 30 d. Indeed, this high-dose tissue concentration at 120 d was similar to the tissue concentrations of fish treated with the medium dose from both study durations. This likely was due to a much lower than expected feed concentration of DDE for the high dose (50 mg/kg), which did not differ in concentration from the medium dose (45.9 mg/kg) for the 120-d exposure study. The lack of significant differences in dietary chemical concentrations be-

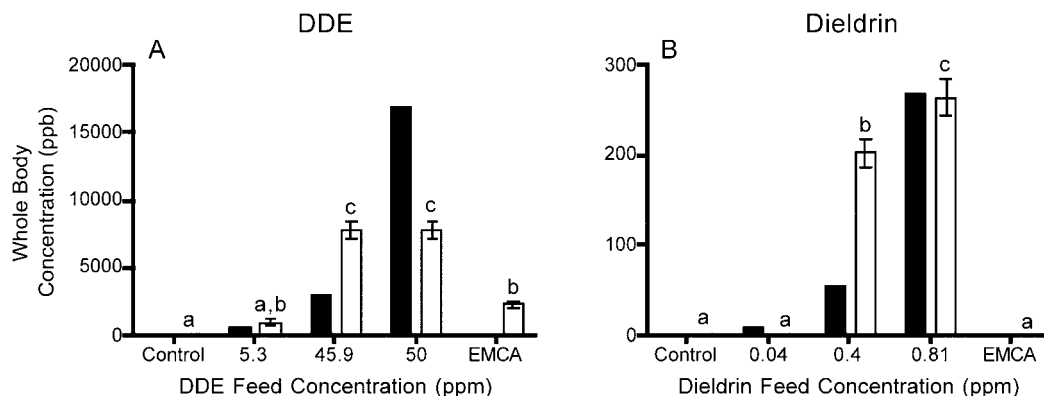


Fig. 2. Mean ( $\pm$  standard error) whole body dichlorodiphenyldichloroethane (DDE) (A) and dieldrin (B) concentrations in fish exposed orally for 120 d ( $n = 3$  per treatment) and fish collected at Emerald Marsh Conservation Area 7 (EMCA;  $n = 5$ ). Treatments with the same lowercase letter were not significantly different ( $p > 0.05$ ). ■ = target whole body; □ = whole body.

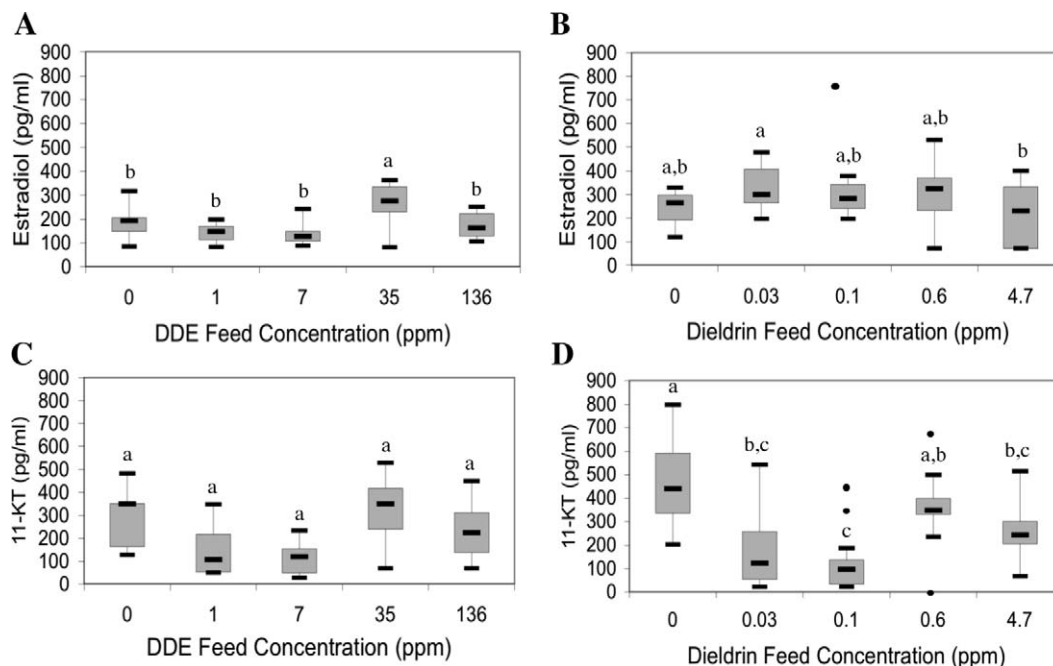


Fig. 3. Box and whisker plots of male (A, B) plasma estradiol and (C, D) 11-ketotestosterone (11-KT) concentrations at day 30 for dichlorodiphenyl-dichloroethane (DDE) and dieldrin, ( $n = 10$  per treatment). The center horizontal line = median value; top and bottom of boxes = 75th and 25th percentiles, respectively; top and bottom of the whiskers = maximum and minimum values; black circles (●) = outliers. Treatments with the same lowercase letter were not significantly different ( $p > 0.05$ ).

tween the medium and high doses for the 120-d exposure likely was due to a dilution error during feed preparation. Dichlorodiphenyldichloroethane and dieldrin concentrations in whole carcasses from EMCA Area 7 were not significantly different from those treated with 1.06 and 7.39 ppm DDE for 30 d, 5.3 ppm DDE for 120 d, and 0.03 and 0.1 ppm dieldrin for 30 d. No dieldrin was detected in fish collected from the EMCA for comparison to the 120-d exposure.

#### Reproductive parameters: Thirty-day exposure

Of the females treated with DDE for 30 d, 72% were in late vitellogenesis, 24% in midvitellogenesis, and the remaining 4% in early vitellogenesis. Of the females treated with dieldrin for 30 d, 80% were in late vitellogenesis, 10% in midvitellogenesis, and the remaining 10% in early vitellogenesis. Of the females treated with the control diet for 30 d, 65% were in late vitellogenesis, 22% in midvitellogenesis, and the remaining 13% in early vitellogenesis. One hundred percent of males (control and treated) had testes showing a high degree of proliferation and sperm maturation. Gonadosomatic index did not differ significantly between replicates or treatments, with the exception that gonadosomatic in females treated with 1.06 ppm DDE for 30 d was significantly higher ( $4.26 \pm 1.87\%$ ) than the control treatment ( $2.05 \pm 0.96\%$ ).

Figures 3 and 4 display box plots showing both the variation and the relationship between feeding dose and circulating hormone concentration in male and female bass, respectively, exposed for 30 d. The variation in hormone measurements appeared greater for female than for male largemouth bass. No relationship was found between chemical dose and plasma hormone concentrations in bass.

#### Reproductive parameters: One hundred and twenty-day exposure

The majority of female largemouth bass treated for 120 d were previtellogenic at day 0 (November), early vitellogenic

at days 30 and 60 (December–January), and 100% were in late vitellogenesis at days 90 and 120 (February–March). No difference was detected in female gonadal stage between fish treated with DDE, dieldrin, or control diets.

No clear dose-response relationships were evident in male or female hormone measurements at day 120 of exposure (Figs. 5 and 6). There appeared to be an increasing dose-response for estradiol versus DDE (Fig. 5A), and a slightly decreasing dose-response for 11-KT versus dieldrin (Fig. 5D) for male largemouth bass. However, the data did not fit the Hill model nor linear regression analyses within statistical bounds due to the high variance in the hormone concentrations among fish within the same treatments. The day-120 hormone data for female largemouth bass demonstrated a similar variability to that of males. The relationships between dieldrin and female plasma concentrations of estradiol and male concentrations of 11-KT (Fig. 6) are suggestive of a dose-related decrease, but again, the data were too variable to provide a statistically reliable dose-response fit. Nonetheless, the data suggest that in males there is a threshold level of dieldrin for decreases of 11-KT (i.e., only the highest doses were affected) and that, in females, all doses of dieldrin resulted in the maximum level of decrease for plasma estradiol.

All endpoints also were evaluated at 30, 60, and 90 d (data not shown; for these time points and results see K.G. Johnson, MS thesis, University of Florida, Gainesville, FL, USA). Similar to the hormone dose-response data collected at 120 d, the 30-, 60-, and 90-d data did not show any consistent dose-response patterns for either sex. However, results did demonstrate clear seasonal trends for plasma hormones in largemouth bass over the 120 d of exposure, as expected and shown previously [9], with increased plasma estradiol and 11-KT in male and female bass, respectively. Although some responses were detected for exposures at 120 d, reproductive success (i.e., egg number, fertility, and hatchability) did not change

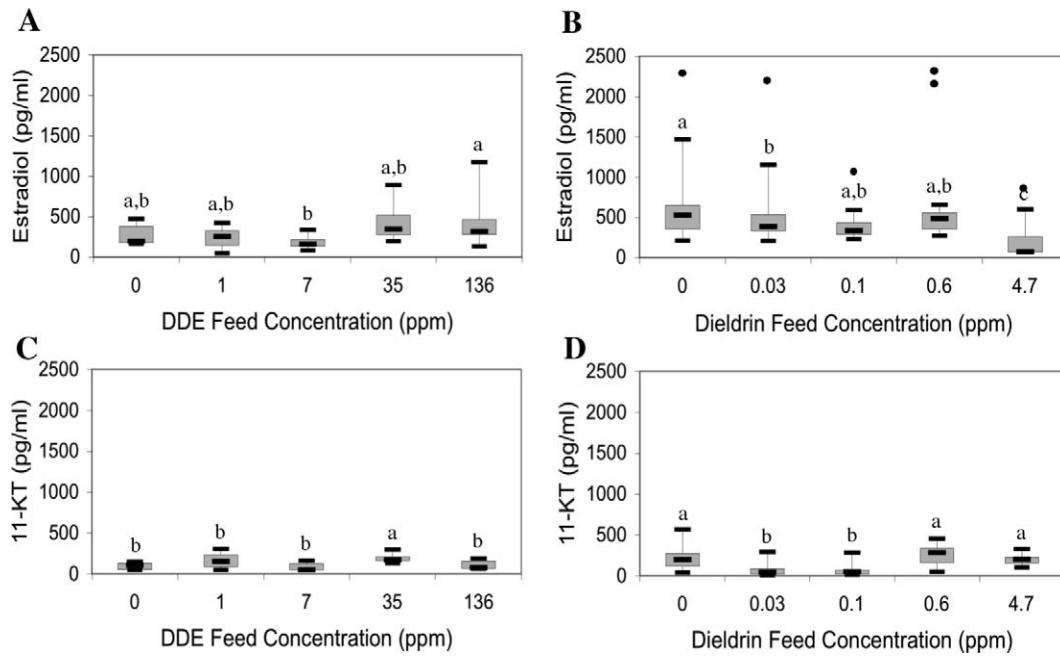


Fig. 4. Box and whisker plots of female (A, B) plasma estradiol and (C, D) 11-ketotestosterone (11-KT) concentrations at day 30 for dichlorodiphenyldichloroethane (DDE) and dieldrin, ( $n = 10$  per treatment). The center horizontal line = median value; top and bottom of boxes = 75th and 25th percentiles, respectively; top and bottom of the whiskers = maximum and minimum values; black circles (●) = outliers. Treatments with the same lowercase letter were not significantly different ( $p > 0.05$ ).

with dose or treatment. Indeed, the percentage of hatched embryos from the spawning event post-120 d of exposure showed no clear dose-dependence, although limited differences between treatments or doses were detected (Fig. 7). These results do indicate that changes in hormone concentrations are not necessarily predictive of apical effects, such as egg hatchability and gonadal stage.

## DISCUSSION

Dietary exposure of largemouth bass to DDE and dieldrin resulted in measurable tissue concentrations, however, large differences were noted between the 30-d and 120-d exposures that cannot be explained by duration of exposure alone. Consumption of high dose feed of either chemical for 30 d leads

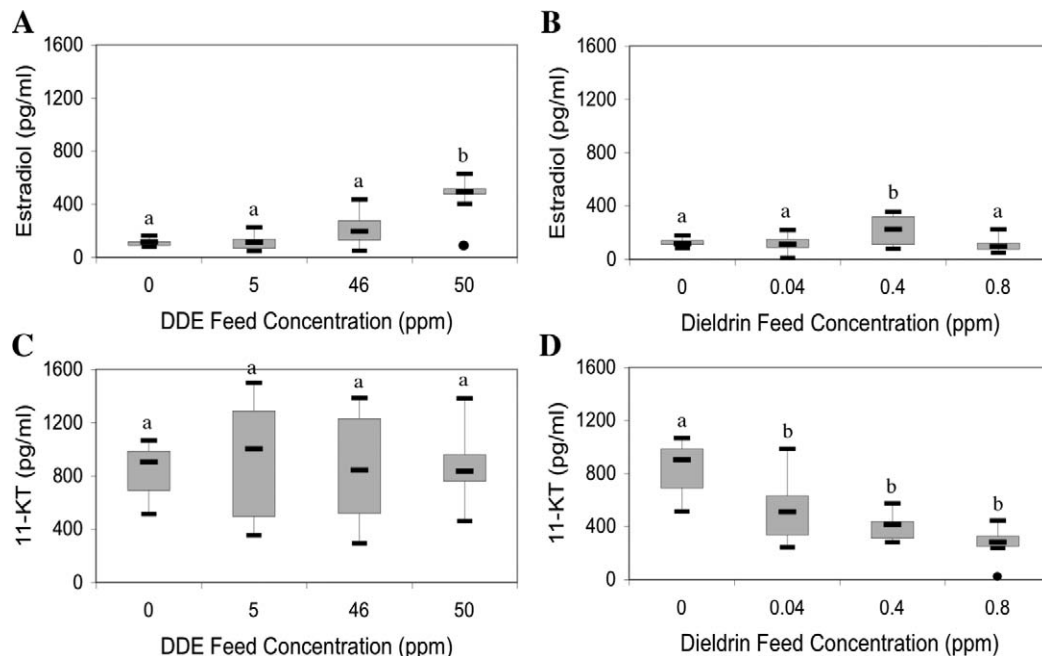


Fig. 5. Box and whisker plots of male (A, B) plasma estradiol and (C, D) 11-ketotestosterone (11-KT) concentrations at day 120 for dichlorodiphenyldichloroethane (DDE) and dieldrin, ( $n = 5$  to 6 per treatment). The center horizontal line = median value; top and bottom of boxes = 75th and 25th percentiles, respectively; top and bottom of the whiskers = maximum and minimum values; black circles (●) = outliers. Treatments with the same lowercase letter were not significantly different ( $p > 0.05$ ).

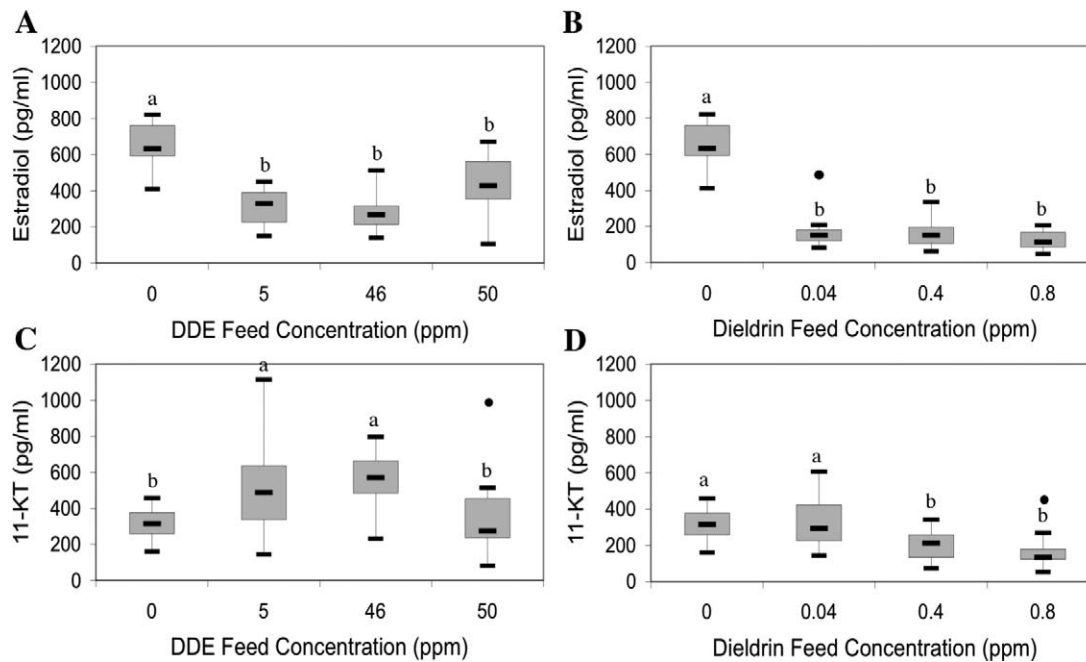


Fig. 6. Box and whisker plots of female (A, B) plasma estradiol and (C, D) 11-ketotestosterone (11-KT) concentrations at day 120 for dichlorodiphenyldichloroethane (DDE) and dieldrin, ( $n = 5$  to 6 per treatment). The center horizontal line = median value; top and bottom of boxes = 75th and 25th percentiles, respectively; top and bottom of the whiskers = maximum and minimum values; black circles (●) = outliers. Treatments with the same lowercase letter were not significantly different ( $p > 0.05$ ).

to high tissue concentrations (~40,000 ppb DDE and 300 ppb dieldrin). The doses achieved after 30 d were higher than would have been predicted from preliminary studies [3] and may have been because animals began consumption of pesticide-laden feed at a more advanced reproductive age in the current study;

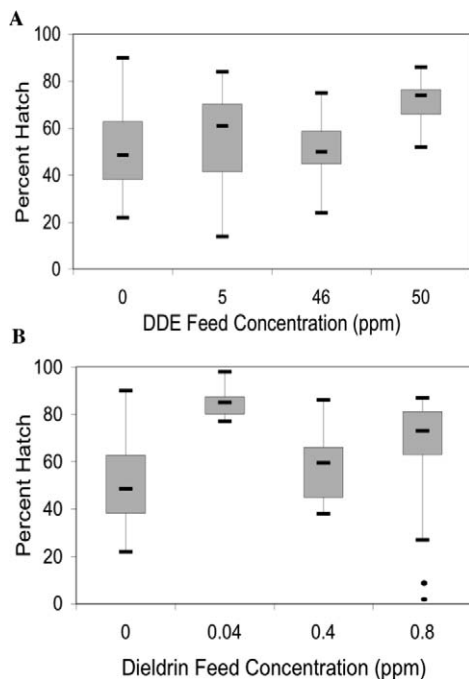


Fig. 7. Box and whisker plots of day 120 percent hatch from fish treated with (A) dichlorodiphenyldichloroethane (DDE) or (B) dieldrin ( $n = 6$  clutches per treatment). The center horizontal line = median value; top and bottom of boxes = 75th and 25th percentiles, respectively; top and bottom of the whiskers = maximum and minimum values; black circles (●) = outliers. Treatments with the same lowercase letter were not significantly different ( $p > 0.05$ ).

female fish accumulate fat during the reproductive season and therefore had a greater percentage of lipid mass available for partitioning of OCPs. The animals fed the medium and highest doses for 120 d developed tissue concentrations much lower than their target and the concentrations found in fish fed high doses for 30 d, again possibly because they were fed throughout the reproductive life-stages, were found with a lower average percentage of lipids in the gonadal tissues. Alternatively, fish fed such high doses of OCPs for a long duration possibly could compensate physiologically for continuous high-level exposure due to a potential saturation of elimination processes. Studies in humans, rats, dogs, and fish all have demonstrated that a steady state (intake = elimination) can be reached with DDE and dieldrin [13–19]. Both DDE and dieldrin have been shown to induce various biotransformation enzymes, possibly increasing their own metabolism when present at high doses [1,20–23].

When studying animals that reproduce annually, timing of the exposure period is of great importance. In Florida, largemouth bass begin sexual maturation in early fall (around October). Hormone concentrations and gonadosomatic in male and female bass, as well as vitellogenin concentrations in females, begin to rise at this time and peak in January or February [9,24]. Spawning usually occurs from February into April, depending on the water temperature.

The 30-d exposure began at the beginning of March, which most likely was too late in the reproductive cycle for any exogenous chemical to alter reproductive function because hormone concentrations likely already had peaked and were declining naturally. When exposure occurred throughout the ovarian cycle (November through March), only effects on sex steroid profiles were detected. Although hormone concentrations appeared to decrease with increasing exposure levels, dose-response relationships were not substantiated by a statistical fit of the data to the Hill model or linear regression

analyses. In some circumstances, we were able to obtain estimates of the dose-response model parameters; however, the statistical uncertainty was so large as to make the results unusable for determining thresholds or for future use in designing mixture experiments. Overall, the variability in the reproductive parameters made dose-response modeling very difficult.

Nonetheless, some responses of potential importance were noted. Indeed, the 120-d exposure study data indicated doses for significant decreases in plasma estradiol when females were exposed to dieldrin and a threshold level for significant decreases in plasma 11-KT in dieldrin-exposed males. These data suggest dieldrin can induce effects similar to depressed sex steroid concentrations in environmentally exposed fish sampled from the EMCA, but at a higher exposure concentration than reported for the EMCA. Future research could investigate the expansion of treatments to completely cover the entire annual reproductive cycle more closely simulating the exposure conditions experienced by largemouth bass in the EMCA. In addition, exposures in fish prior to sexual maturation also may provide useful information on effects resulting from life-time exposure.

These studies only sought to characterize single chemical dose-response effects for two of the predominate OCPs, *p,p'*-DDE and dieldrin, found in the soils and various tissues of largemouth bass from the EMCA. Fish in this system are exposed to multiple pesticides (e.g., both toxaphene and chlordane in addition to DDE and dieldrin) that may not only contribute to reductions in hormone concentrations, but also to decreased reproductive success. Future research on the reproductive effects of OCPs on largemouth bass also could focus on pesticide mixture exposures that more closely simulate natural conditions where depressed endocrine function and reproductive success have been observed. The application of studies using multiple single chemical doses, coupled with mixture exposures, may enable researchers to ascertain whether OCPs are responsible for modulating endocrine function in these areas.

**Acknowledgement**—The authors would like to thank J. Scarborough, J. Grosso, J. Wiebe, K. Kroll, D.E. Canfield, Jr., C.E. Cichra, and R.H. Rauschenberger. Funding was provided by the American Chemistry Council (grant END0020) to C.J. Borgert and T.S. Gross, and the National Institute of Environmental Health Sciences (Superfund project P42 ES07375) to T.S. Gross.

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