SPECIAL PUBLICATION SJ2009-SP5

AN EVALUATION OF THE BIOACCUMULATION OF ORGANOCHLORINE PESTICIDES IN GREAT EGRETS (*CASMERODIUS ALBUS*): LABORATORY MODEL FOR THE NORTH SHORE RESTORATION AREA AT LAKE APOPKA

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DATA REPORT:

An Evaluation of the Bioaccumulation of Organochlorine Pesticides in Great Egrets (*Casmerodius albus*): Laboratory Model for the North Shore Restoration Area at Lake Apopka

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Acknowledgements

We would like to acknowledge Edgar Lowe, Michael Coveney, Roxanne Conrow, Erich Marzolf, Elizabeth Mace, Steven Richter, and Pamela Bowen, (St. Johns River Water Management District, Division of Environmental Sciences, Department of Water Resources, Palatka, FL) for their invaluable assistance in designing and overseeing the progress of this project. We would like to thank staff members from the joint University of Florida - USGS-Ecotoxicology Program (Gainesville, FL) for their invaluable help in the maintenance and rearing of great egrets. Special thanks to Janet Scarabough, Jon Wiebe, Travis Smith, and Carla Wieser. In addition, we would like to thank Dr. Denise Gross for her veterinary expertise and health assessments of birds. This study was funded by the St. Johns River Water Management District through a four year grant to Timothy S. Gross.

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Abstract

During 1998-1999, a significant mortality of several piscivorous bird species occurred on the former agricultural property on the north shore of Lake Apopka (North Shore Restoration Area, NSRA), Florida. Although this mortality was ultimately linked to organochlorine pesticide (OCP) toxicosis, there was very little understanding of the rate of bioaccumulation of OCPs by fish-eating birds feeding on these flooded sites. Studies were conducted in three phases during 2002 through 2004: Phase I - to determine the potential for bioaccumulation of organochlorine pesticides (OCP) in great egrets (*Casmerodius albus*) fed diets consisting of primarily blue tilapia (*Oreochromis aurea*) exposed to OCP-contaminated soils/sediments from the NSRA and evaluate health and growth effects; Phase II – to determine the potential for toxicosis from NSRA-derived OCPs in great egrets; and Phase III – to evaluate the effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets. These feeding studies were conducted during the spring and summer of three consecutive years (2002 to 2004). For all years, egrets were collected as nestlings (< 4 weeks of age) from wild colonies located in either south or north-central Florida and were raised in the laboratory to juvenile age (~ 2 months of age) prior to the start of OCP-fish dosing. Dosing was achieved by feeding egrets ground OCP-contaminated fish sausage in natural casings. During 2002 (Phase I), ten birds were fed a diet consisting of approximately 40% NSRA-OCP-contaminated tilapia sausage. Five birds were euthanized at 8 weeks and five at 11 weeks to evaluate bioaccumulation of OCPs. During 2003 (Phase 2), ten birds were dosed as follows: three controls and 7 treated (fed 100% NSRA-OCP-contaminated tilapia sausage for 8 weeks). Four treated egrets exhibited signs of poor health or potential toxicosis by 7 weeks and were euthanized (symptoms included lethargy, decreased food intake, tremors, inability to perch and poor mobility), and the remaining egrets were euthanized at 8 weeks as per the experimental design. During 2004 (Phase III), thirty birds were split into two groups and either fed/dosed with 100% diet of NSRA-OCP-contaminated fish (n=22) or control fish (n=8) for 18 to 20 weeks. NSRA-OCP fed birds were sacrificed at three week intervals (weeks 3, 6, 9, 12, 15 and 18) through week 18 to assess bioaccumulation over time. At the conclusion of week 18, the remaining birds (n=10) were maintained for two additional weeks and sacrificed as follows: n=3 NSRA-OCP birds maintained as treated (week 20 treated egrets); n=3 control birds maintained on control diets (week 20 control egrets); and n=4 NSRA-OCP birds which were starved (provided no food but water provided ad libitum; week 20 starved birds) to assess effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets. Results demonstrated significant bioaccumulation of OCPs in egrets from dietary intake of NSRA-tilapia through 18 weeks. Results demonstrated that a 100% diet of NSRA tilapia was capable of a resultant toxicosis, which could, in part, explain the 1998-99 avian mortality event reported for the NSRA. Results also indicated that seasonal and/or health derived reductions in dietary intake would repartition OCPs and that this mechanism may be a critical component in the toxicosis of OCPs in wading birds.

Introduction

During the fall of 1998 and winter of 1999, a significant bird mortality event occurred on the former agricultural properties on the north shore of Lake Apopka, an area now known as the North Shore Restoration Area (NSRA). The US Fish and Wildlife Service suggested that this mortality was linked to organochlorine pesticide (OCP) toxicosis.

A primary uncertainty for evaluating risk from OCP pesticide residues is the rate of bioaccumulation of OCPs from soils/sediments through the food chain to fish and then to birds. Pesticides of potential concern in the NSRA include toxaphene, DDT and its metabolites DDE and DDD, chlordanes, and dieldrin. In addition, the NSRA soils contain a high level of organic matter, which would enable a high OCP burden. Areas with similar high organic matter contents have not been studied, nor has environmental fate modeling for OCPs been evaluated in these or similar high organic conditions. Hence, it was unclear whether the magnitude of bioaccumulation reported for other extensively studied areas could be directly and validly applied to the NSRA and therefore, whether risk assessments for the NSRA would be valid. In addition, there was little data on the bioaccumulation of toxaphene in wildlife. Therefore, three phases of study were necessary initial components of restoration efforts for the NSRA: 1) laboratory microcosms, 2) field-scale mesocosms, and 3) bird feeding studies.

Previous ecological risk assessments have utilized the biota-sediment accumulation factor (BSAF) to estimate the bioaccumulation of OCP from sediments into the food chain, including fish and ultimately birds. Indeed, macroinvertebrates, amphibians, and fish serve as important and critical links in the transfer of sediment-associated chemicals to higher trophic levels, such as birds. The BSAF is a relatively simplistic environmental fate model that characterizes the bioaccumulation of lipophilic OCPs as a function of organic carbon in sediments and percent of lipid in aquatic organisms. However, little data existed for the primary OCPs and at the high organic content found in the NSRA soils. Both microcosm and mesocosm studies were conducted to enable an assessment/determination of site-specific BSAFs for OCPs from soil/sediment in the NSRA. In addition, a series of three studies with great egrets was also conducted to better understand the bioaccumulation of

OCPs by fish-eating birds inhabiting flooded marshes. This data report summarizes and describes these three efforts with great egrets and includes all raw results.

Study Objective

Determine the bioaccumulation of organochlorine pesticides (OCPs) in great egrets (*Casmerodius albus*) from diets representing potential NSRA exposure.

Specific Aims

The overall specific aims for this study were:

1. Determine the potential for bioaccumulation of organochlorine pesticides (OCP) in great egrets (*Casmerodius albus*) fed diets consisting of primarily blue tilapia (*Oreochromis aurea*) exposed to OCP-contaminated soils/sediments from the North Shore Restoration Area at Lake Apopka Florida.
2. Determine the effects of OCPs on growth, food intake, and hematological and histopathological parameters.
3. Evaluate the effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets.

Materials and Methods

Phase I: 2002

Summary of Experimental Design: Fifteen great egret nestlings were collected from south Florida in spring of 2002. Three birds were euthanized following approximately 30 days of captivity (6/4/2002) and samples were collected for baseline contaminant analyses (day -90 controls), and an additional bird was sacrificed at both day -55 (control) and day -21 (control) for analyses of baseline contaminants (see Table 1 summary). Treatment day was listed as days from the initiation of feeding the NSRA-OCP diet (see Table 2). The remaining ten birds were split into two groups and fed/dosed with the NSRA-OCP-contaminated fish for either eight (56 days, Group A) or 11 (74 days, Group B) weeks. During the course of the dosing experiment, birds were fed an average of 105 ± 58 g of fish/day (~ 8 % body weight) of a diet consisting of ~ 40% NSRA-OCP-contaminated fish derived sausages and 60% clean fish as explained in more detail below. Food consumption was recorded daily. Every two weeks, birds were weighed and measured to monitor growth and assess general condition and health, and whole blood was collected to assess OCP concentrations (see Table 2 summary). At the end of the experiment, birds were necropsied and whole blood, brain, liver, abdominal fat, feathers, and remaining carcass were collected for OCP analyses.

Source of Birds: Fifteen great egret nestlings were collected from a colony located in South Florida, Water Conservation Area 3 (N 25°57.330', W 080°33.920') on May 6, 2002. Attempts were made to collect only one nestling per nest, and this was accomplished with the exception of two nests where more than one nestling was collected. Immediately after collection, nestlings were ground-transported in heated containers/coolers to the USGS/Florida Integrated Science Center/Center for Aquatic Resource Studies (USGS/FISC/CARS), Gainesville, FL (see photos below). A summary of body sizes for birds included in this study is presented in Table 3. Based on these values, it was estimated that nestlings had hatched approximately 5 to 15 days prior to collection. All birds were initially maintained on a diet of clean fish (capelin and silversides) which were submitted for

OCP analyses to ensure low or background OCP concentrations (Table 4). In addition, three birds (control/treatment day -90) were submitted to the laboratory for a complete OCP screen to assess baseline body burdens and the results from these analyses are presented in Table 5.

Housing and Maintenance of Nestlings:

Immediately after arrival to the laboratory, great egret nestlings were placed in individual 120 L clear plastic containers lined with absorbent bench underpads and fitted with artificial stick nests (see photos below). Containers (0.7 m^2) were placed inside a walk-in incubator and air temperature and humidity maintained at 30°C and 75%, respectively. After one to two weeks post-arrival and depending on the initial size of nestlings, birds were moved to larger fiberglass tanks (4.0 m^3) which were placed indoors in a wet laboratory. Tanks were slanted so that one end of the tank always had access to standing water (~5 cm) while the other end was always kept dry and had an artificial stick nest. All tanks were cleaned daily and sticks were cleaned and/or replaced every other day. During this period, nestlings were moved outdoors for approximately 30-45 minutes/day and placed in 2,600 L plastic round tanks with other egrets so they could get exposure to natural sunlight. Tanks were filled with a small amount of water (5 cm) and contained large sticks to perch on. Egrets were fed twice a day a diet consisting of ~ 50 g/bird/day of chopped capelin (*Osmerus villosus*) and silversides (*Menidia* spp.) mixed with a ThiaminE supplement (1 ml/kg fish) as well as a multi-vitamin supplement (Mazuri® Vita-Zu Bird tablet, 1 tablet per 1 kg/fish). Fish were purchased from a commercial source (McRoberts Fish Supply) and arrived as frozen blocks. To ensure that birds were fed a non-contaminated diet, prior to the start of the experiment a random sample of seven whole fish composites were analyzed for a complete OCP suite (En Chem now Pace Analytical Services Inc., Green Bay, WI 54302). Fish were found to contain no significant OCP residues as shown in Table 4. As they outgrew the fiberglass tanks in the wet lab, egrets were moved to outdoor facilities after approximately three weeks or body size of ~ 700 g after arrival to the USGS.

Housing and Maintenance of Fledglings and Juveniles:

Egrets were moved to outdoor chain linked enclosures when they reached a minimum size of ~ 700 g. These enclosures had a floor space of 65 m^2 and a height of 128 cm and were constructed on top of a concrete basin (see photos below). Enclosures had multiple access gates (three on each side) and were covered with a clear roof. Each enclosure also had running water (depth of < 3 cm), multiple perches, and standing blocks above the water line for birds to rest upon and for positioning food trays. At this stage, birds were fed the same diet as during the nestling ages but at a rate of 75 g/bird/day. In addition, birds were also acclimated or trained during this period to feeding on "fish sausages". Fish sausages were made by grinding the fish with an industrial Hobart grinding machine and stuffing this content into natural pork or sheep intestine casings (see photos below). Casings (n = 2) were also analyzed for OCPs and found to contain background concentrations of OCPs. Sausages were weighed individually (25 ± 1g each), tied at both ends with a suture knot, and stored at -20°C until the day before they were fed to the birds. Egrets were maintained on this diet until the start of the dosing study, at which point fish sausages were produced containing a mixture of NSRA-OCP- contaminated blue tilapia (*Oreochromis aurea*) and control fish instead. A summary of food consumption rates by birds prior to the start of OCP dosing is presented in Figure 1.

Dosing of Egrets with OCP-contaminated Fish Diets: On September 3, 2002, birds were split into two groups, and dosed with NSRA-OCP-contaminated tilapia sausage for either eight (56 days; Group A) or 11 (74 days; Group B) weeks. Throughout this period, birds were fed an average of 105 ± 58 g of fish/day (~ 8 % body weight) of a diet consisting of ~ 40% OCP-contaminated blue tilapia sausages and 60% clean fish (silversides and capelin). A summary of food consumption rates by bird and type of feed is presented in Figure 2. Blue tilapia and sunfish were "naturally" exposed to OCPs after stocking a clean population of fish into two 0.1 ha earthen ponds which were constructed on the NSRA. Specifically, fish ponds were constructed by the St. Johns River Water Management District (SJRWMD) , on one field of the former Hooper Farm. This field is known to contain soils high in OCPs (n = 16, mean ± SD for dieldrin= 1,121 ± 324; DDE= 4,634 ± 1386; DDD= 1,456 ± 324; DDT=433 ± 192; total DDT= 6,523 ± 1204; *alpha*-chlordane= 841 ± 228; *gamma*-

chlordane=533 ± 116, and toxaphene=44,500 ± 16,071 µg/kg dw) and total organic content (TOC, 346,563 mg/kg, approx. 35 ± 3.0%). Ponds were flooded and maintained by the SJRWMD at about 1-m depth with water from an adjacent canal. After two months, each pond was stocked with 500 juvenile blue tilapia (approximate size of 3 - 8 cm in length) and shortly thereafter with crayfish, mosquitofish and sunfish. Fish were harvested by the SJRWMD with seines from the ponds after 2 months of bioaccumulation since previous studies had shown fish attained steady state after this time (SJRWMD Report: SJ2005-SP11, Microcosm Evaluation of the Bioaccumulation of Organochlorine Pesticides From Soils in the NSRA at Lake Apopka). Both tilapia and sunfish were captured with seine hauls. However, since over 80 percent of the biomass removed was tilapia we refer in this report to the harvested fish as "tilapia". Un-harvested fish remaining in the ponds reproduced to provide sufficient biomass for the subsequent collections throughout all phases of the study. Tilapia were brought to the laboratory, washed of excess mud and debris, ground, thoroughly mixed for approximately 1.5 hours, and made into sausages as already described. The ground fish sausage mix was divided up into several batches and frozen at -20ºC. Sausages were then made in batches large enough to feed birds for two weeks at a time. A total of 5 random grab samples of this ground tilapia mix was sent to the laboratory and analyzed for OCP concentrations. Results of these analyses are presented in Table 6.

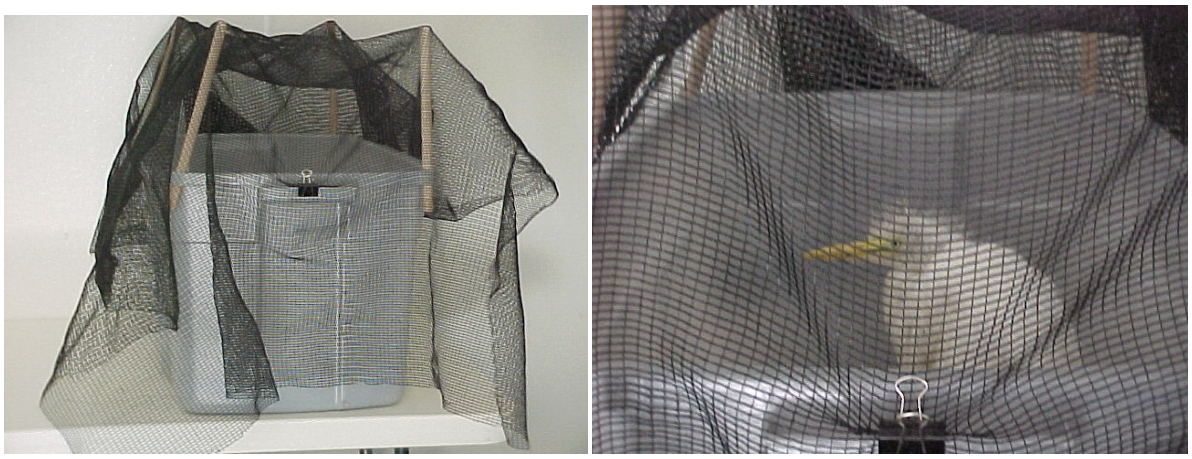
Endpoints: Daily food consumption was determined by weighing the amount of food offered and subtracting any remains left behind. Food was served in disposable plastic weigh dishes, and any uneaten food left in the tray or in the enclosure floor was collected and weighed. Growth of egrets was determined by weighing and measuring (bill and tarsometatarsus length) each bird at a frequency of once a week until start of dosing experiment (all measurements), to approximately every two weeks thereafter (body weight only). Birds were weighed inside a mesh bag using a pesola scale and measured using calipers. Blood samples (1 – 3 mL) were taken from the jugular vein using 3 mL syringes fitted with 24 11/2" G needles and stored in cryovials at -20°C for later OCP analysis. At the end of the experiment, birds were euthanized by cervical dislocation as per University of

Florida IACUC guidelines, and several tissues (brain, liver, abdominal fat, primary feathers, and remaining carcass) weighed and frozen at -20°C for later OCP analyses.

Statistical analyses:

Data were analyzed for descriptive statistics (mean, SE, SD etc) and treatment differences using the Statistical Analysis System (SAS), version 9. Treatments and time differences were analyzed using an analysis of variance (ANOVA) and differences detected using Dunnet's Multiple Range test as a multiple comparison of means procedure analysis.

Photographs 1: Collection of nestlings (A and B): hatchlings were transferred from south Florida collection sites to laboratory facilities in Gainesville FL in insulated containers. Egret hatchlings were maintained for initial one to two weeks in nest boxes/containers (C) and were exposed to natural light and other nestlings every other day (D).



Photographs 2: Maintenance of egret hatchlings during first 2 weeks in nest boxes

Photographs 3: Production of fish sausages. Fish were initially ground and stored as a single composite of NSRA fish and control fish prior to sausage production and to ensure batch consistency (A). Sausages were produced by re-grinding and extrusion into sheep intestinal casing (B).

Photographs 4: Maintenance of egret fledgling through adult size stages utilized outdoor concrete and chain link enclosures. Each animal was housed individually.

Phase II: 2003

Summary of Experimental Design:

Eighteen great egret nestlings were collected from South Florida Water Conservation Area #3 on 4/22/2003 (birds # 1-18). Birds from this collection had several medical problems, including severe parasite loads and metabolic bone disease that necessitated that they not be utilized for this experimental trial and were therefore euthanized. An additional 15 great egret nestlings were collected on 4/28/2003 from a colony located at Gatorland alligator farm. The original study design called for 24 egrets, divided into four OCP dietary treatments (control, low, medium, and high OCPs) of six birds each, with an extra six birds collected to replace any possible losses. However, because of the much higher than expected mortality of birds, the study had to be re-designed to include only the surviving birds (n = 10). Three birds were not dosed and tissues collected for background contaminant analyses either prior to the start of OCP dosing (n=1) or at the conclusion of dosing (n=2), whereas the remaining seven birds were assigned to a dietary dosing for 8 weeks with OCP-contaminated fish sausage. However, one egret sustained significant wing and leg injuries at Week 4 and was euthanized; four egrets exhibited signs of poor health or potential toxicosis by 7 weeks and were euthanized (symptoms included lethargy, decreased food intake, tremors, inability to perch and poor mobility), and the remaining three egrets were euthanized at 8 weeks as per the experimental design (see Table 7 and 8). During the course of the dosing experiment, birds were fed an average of 95 ± 3.0 g of fish sausage/day (~ 8 % body weight) of a diet consisting of ~ 100% OCP-contaminated fish sausages. Food consumption was recorded daily. At the end of the experiment, birds were necropsied and whole blood, brain, liver, abdominal fat, and the carcass remainders were collected for OCP analyses (Table 8). In addition, tissues were preserved in buffered formalin for histopathological evaluations. Blood was also collected at weeks 0, 4, 7 and 8 from all birds for hematological and biochemistry panels.

Source of Birds:

Eighteen great egret nestlings were collected from South Florida Water Conservation Area #3 on 4/22/2003 (birds # 1-18; (N 25°57.330', W 080°33.920')). An additional 15 great egret nestlings were collected on 4/28/2003 from a colony located at Gatorland Alligator Farm (14501 South Orange Blossom Trail, Orlando, FL) in Central Florida (birds #19 – 33). Attempts were made to collect only one nestling per nest, and this was accomplished with the exception of seven nests where more than one nestling was collected. Immediately after collection, nestlings were ground-transported in heated containers/coolers to the USGS/Florida Integrated Science Center/Center for Aquatic Resource Studies (USGS/FISC/CARS), Gainesville, FL. A summary of body sizes for all birds at the time of collection is presented in Table 9. Based on these values, it was estimated that nestlings had hatched approximately 15 to 35 days prior to collection. Background OCP concentrations were assessed in a total of three birds (one "control" bird was euthanized on 6/05/03 prior to the start of OCP dosing, and two "control" birds were euthanized at the end of the experiment on 9/9/03). Results from these analyses are summarized in Table10.

Housing and Maintenance of Birds:

Nestlings, fledglings, and juvenile great egrets were raised as described under Phase I methods above. The primary difference among both years consisted of using hatchery-raised blue tilapia (Southern Fish Culturist, Florida) as a source of clean fish for fish sausages used to train fledglings and juveniles, and also to feed two control egrets throughout the study. Approximately one month prior to the start of the dosing experiment, each bird was offered 150-175 g of clean tilapia sausages (6 - 7 sausages) daily and the same multivitamin additive as described in phase I above. Tilapia were found to contain no significant OCP residues as shown in Table 11.

Dosing of Egrets with OCP-contaminated Fish Diets:

Oral dosing with NSRA-OCP fish began on 7/7/03 when seven birds were fed a diet consisting of 100% OCP-contaminated tilapia for a total of 4 to 8 weeks. Two additional birds were used as controls and were fed fish sausages containing clean blue tilapia. All birds were fed 6 to 8 sausages/day or an average of 80 ± 3.0 g of fish sausage/day (~ 8 %

body weight). A summary of food consumption rates is presented in Figure 3. Blue tilapia were "naturally" exposed to OCPs after stocking a clean population of fish into two 0.1 ha earthen ponds which were constructed on the NSRA as described under Phase I methods. Blue tilapia were harvested, brought to the laboratory, washed of excess mud and debris, ground, thoroughly mixed for approximately 1.5 hours, and made into sausages as already described. Sausages were made in four large batches. A total of 4 random grab samples of this ground tilapia mix was sent to the laboratory and analyzed for OCP concentrations. Results of these analyses are presented in Table 12.

Endpoints: Daily food consumption was determined by weighing the amount of food offered and subtracting any remains left behind. Food was served in disposable plastic weigh dishes, and any uneaten food left in the tray or in the enclosure floor was collected and weighed. Growth of egrets was determined by weighing and measuring (bill and tarso-metatarsus length) each bird at a frequency of once a week until two months prior to the start of the dosing experiment (all measurements) and at the end of the dosing study (body weight only). Birds were weighed inside a mesh bag using a pesola scale and measured using calipers. Blood samples (1 – 3 mL) were taken from the jugular vein using 3 mL syringes fitted with 24 11/2" G needles and stored in cryovials at -20°C for later OCP analysis. At the end of the experiment, birds were euthanized by cervical dislocation as per University of Florida IACUC guidelines, and several tissues (brain, liver, abdominal fat, primary feathers, and remaining carcass) weighed and frozen at -20°C for later OCP analyses. Tissues were also fixed in 10% buffered formalin for histopathological evaluations.

Hematology and Blood Chemistry

Blood chemistry and hematology were utilized to assess health or disease status. Blood chemistry analyses included: anion gap, plasma albumin, alkaline phosphatase (ALK), aspartate aminotransferase (AST), calcium, carbon dioxide, chloride, creatinine kinase, globulin, glucose, magnesium, phosphorous, plasma proteins, potassium, sodium, and uric acid. Chemistry parameters were utilized as indicators of liver, kidney and metabolic function. Blood hematology analyses included: hemoglobin, packed cell volume (PCV), red

blood cells (RBCs), white blood cells (WBCs), mean corpuscular volume (RBC size; MCV), mean corpuscular hemoglobin (hemoglobin per RBC; MCH), mean corpuscular hemoglobin per cell (hemoglobin per RBC size; MCHC),and other blood cell types including heterophils, lymphocytes, monocytes, eosinophils, and basophils (each listed as numbers of cells per volume and % of total cells. Both blood chemistry and hematology were evaluated for treatment (OCP exposure) effects.

Statistical analyses:

Data were analyzed for descriptive statistics (mean, SE, SD etc) and treatment differences using the Statistical Analysis System (SAS), version 9. Treatments and time differences were analyzed using an analysis of variance (ANOVA) and differences detected using Dunnet's Multiple Range test as a multiple comparison of means procedure analysis.

Phase III: 2004

Summary of Experimental Design:

Thirty-three great egret nestlings were collected from North Central Florida during spring 2004. Three birds were euthanized following approximately 20 days of captivity (5/28, 6/1, and 6/3/2004) and samples collected for baseline contaminant analyses. Treatment day was listed as days from the initiation of feeding the NSRA-OCP diet (Table 13). The remaining thirty birds were split into two groups and either fed/dosed with the NSRA-OCP-contaminated fish sausage (n=22) or control fish sausage (n=8) for the remainder of this experimental trial. During the course of the dosing experiment, birds were fed an average of 125 ± 43 g of fish sausage/day (~ 8 % body weight) of a diet consisting of 100% NSRA-OCP-contaminated fish derived sausages. Food consumption was recorded daily. NSRA-OCP fed birds were sacrificed at three week intervals (weeks 3, 6, 9, 12, 15 and 18) through week 18 (see Table14 for summary) to assess bioaccumulation over time. At the conclusion of week 18, the remaining birds were sacrificed as follows: n=3 NSRA-OCP birds were maintained for two additional weeks on NSRA-OCP diets prior to sacrifice (Week 20 treated egrets); n=3 control birds maintained on control diets for two additional weeks prior to

sacrifice (week 20 control egrets); and n=4 NSRA-OCP birds were starved (provided no food but water provided ad libitum) for two additional weeks prior to sacrifice (week 20 starved birds) to assess effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets. At the end of the experiment, birds were necropsied and whole blood, brain, liver, abdominal fat, subcutaneous fat and carcass remainders were collected for OCP analyses. Blood was also collected for the conduction of hematological and biochemistry panels (Table 14).

Source of Birds:

Thirty-three great egret nestlings were collected from 10 nests within a colony located in north central Florida, at Lake Lochloosa on May 5, 2004. Immediately after collection, nestlings were ground-transported in heated containers/coolers to the USGS/Florida Integrated Science Center/Center for Aquatic Resource Studies (USGS/FISC/CARS), Gainesville, FL. A summary of body sizes for birds included in this study is presented in Table 15. Based on these values, it was estimated that nestlings had hatched approximately 18 to 35 days prior to collection. Background OCP concentrations were assessed in a total of three birds (one bird was euthanized on 6/05/03 prior to the start of OCP dosing, and two "control" birds were euthanized at the end of the experiment on 9/9/03). Results from these analyses are summarized in Table 16.

Housing and Maintenance of Birds:

Nestlings, fledglings, and juvenile great egrets were raised as described under Phase I methods above. The primary difference among the three years consisted of using hatchery-raised blue tilapia (Southern Fish Culturist, Florida) as a source of clean fish for fish sausages used to train fledglings and juveniles, and also to feed eight control egrets throughout the study. Approximately one month prior to the start of the dosing experiment, each bird was offered 140-160 g of clean tilapia sausages (6 - 7 sausages) daily and the same multivitamin additive as described above in phases I and II above. Control tilapia were found to contain no significant OCP residues as shown in Table 17.

Dosing of Egrets with OCP-contaminated Fish Diets:

Oral dosing with NSRA-OCP fish sausage began on 6/20/03 when 22 birds were fed a diet consisting of 100% NSRA-OCP-contaminated fish sausage. Eight additional birds were used as controls and were fed fish sausages containing 100% clean blue tilapia. All birds were fed 5 to 7 sausages twice daily or an average of 145 ± 43g of fish/day (~ 8 % body weight). Blue tilapia were "naturally" exposed to OCPs after stocking a clean population of fish into two 0.1 ha earthen ponds which were constructed on the NSRA as described under Phase I methods. Blue tilapia were harvested, brought to the laboratory, washed of excess mud and debris. NSRA tilapia were initially harvested at a mass predicted to be sufficient to complete phase III, however, consumption and body weights were higher than those originally predicted so an additional collection of NSRA tilapia was conducted. Both batches were ground and mixed to prepare a single homogeneous mass for this study phase. Control tilapia were similarly prepared. Ground NSRA and control tilapia were stored frozen and thawed as needed to produce weekly supplies of sausages. Sausages were then made in batches enough to feed birds for at least one week at a time. A total of 15 sausage batches were produced and samples sent to the laboratory and analyzed for OCP concentrations. Results of these analyses are presented in Table 18. Samples 1 through 12 are for first NSRA tilapia collection and samples 13 through 15 for the second NSRA tilapia collection.

Endpoints: Daily food consumption was determined by weighing the amount of food offered and subtracting any remains left behind. Food was served in disposable plastic weigh dishes, and any uneaten food left in the tray or in the enclosure floor was collected and weighed. Growth of egrets was determined by weighing and measuring (bill and tarso-metatarsus length) each bird prior to sacrifice. Birds were weighed inside a mesh bag using a pesola scale and measured using calipers. Blood samples (1 – 3 mL) were taken from the jugular vein using 3 mL syringes fitted with 24 11/2" G needles and stored in cryovials at -20°C for later OCP analysis. At the end of the experiment, birds were euthanized by cervical dislocation as per University of Florida IACUC guidelines, and several tissues (brain, liver, abdominal fat, primary feathers, and remaining carcass) weighed and frozen at -20°C for later OCP analyses. Tissues were also fixed in 10% buffered formalin for potential later histopathological evaluations.

Hematology and Blood Chemistry

Blood chemistry and hematology were utilized to assess health or disease status. Blood chemistry analyses included: alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), billirubin, blood urea nitrogen (BUN), calcium, carbon dioxide, cholesterol, creatinine, creatinine kinase, glucose, GGT, lactic dehydrogenase, lipase, phosphorous, plasma proteins, potassium, sodium, triglycerides, and uric acid. Chemistry parameters were utilized as indicators of liver, kidney and metabolic function. Blood hematology analyses included: red blood cells (RBCs), white blood cells (WBCs), hematocrit, and other blood cell types including heterophils, lymphocytes, monocytes, eosinophils, and basophils (each listed as % of total cells). Both blood chemistry and hematology were evaluated for treatment (OCP exposure) effects.

Statistical analyses:

Data were analyzed for descriptive statistics (mean, SE, SD etc) and treatment differences using the Statistical Analysis System (SAS), version 9. Treatments and time differences were analyzed using an analysis of variance (ANOVA) and differences detected using Dunnet's Multiple Range test as a multiple comparison of means procedure analysis.

Results and Discussion

This data report summarizes and describes the results for each of the three phases of the great egret studies presented above. In addition, the appendices include the raw data for these studies.

Phase I (2002) was the initial study which focused on the development and validation of husbandry and feeding/dosing of great egrets under captive conditions, as well as the initial evaluation of bioaccumulation of OCPs in great egrets from NSRA exposed fish. This phase fed at a rate of 40% of total diet consisting of NSRA OCP fish sausage as a conservative approach to assess bioaccumulation rather than adverse effects.

Phase II (2003) attempted to expand these results to include evaluations of OCP bioaccumulation as a function of dose. However, it should be remembered that this phase was minimal in scope due to extensive health issues with egret hatchlings encountered during the initiation of this phase. It must also be noted, that this phase was the "worse-case-scenario" with the diet consisting of 100% NSRA-OCP fish sausage and therefore had the potential for OCP effects as well.

Phase III (2004) was an expansion of these efforts to include a more thorough evaluation of exposure length/time on bioaccumulation. This phase utilized a moderate dose with the diet consisting of 100% NSRA-OCP fish sausage. In addition, this phase also focused on the potential effects of fasting on tissue bioaccumulation and the repartitioning of OCPs in birds that may occur as a normal response to the seasonal decrease in dietary intake in wading birds.

Collectively, the three phases offer the first assessment of weathered OCPs from high organic content soils as well as for toxaphene in wading birds. Results are limited in this report to the primary OCPs found at the NSRA: p,p' DDE, p,p' DDD, dieldrin, toxaphene, *alpha*-chlordane and *gamma*-chlordane, however other OCPs were routinely detected at lower concentrations and these data are included in the appendices. Most importantly, these efforts were designed to elucidate the potential exposures and OCP effects that may have played an important role in the bird mortality events of 1998-99 on the NSRA.

Phase I: 2002

Fifteen great egret nestlings were collected from South Florida in spring of 2002. Body measurements were recorded for all birds immediately following collection to estimate age and condition (Table 3). All birds initially were fed twice a day using a control diet of fish which did not contain significant OCP residues (Table 4). Three birds were euthanized following approximately 30 days of captivity (6/4/2002) and samples were collected for baseline contaminant analyses (day -90 controls), and an additional bird also was sacrificed at both day -55 (control) and day -21 (control) for additional analyses of baseline contaminants (Table 5). Control birds did not have significant tissue/body burdens of OCPs. Treatment day was listed as days from the initiation of feeding the NSRA-OCP sausage diet. The remaining ten birds were split into two groups and fed/dosed with the NSRA-OCP-contaminated fish sausages for either 56 days (Group A) or 74 days (Group B) weeks. This technique of producing and utilizing fish sausages was developed during this phase of study and constituted a unique and novel method for dosing. Birds acclimated well to the sausage diet which enabled the controlled feeding of NSRA-OCP fish to great egrets. Sausages were produced in multiple batches and each analyzed for OCP concentrations (Table 12). Sausage OCP results exhibited significant variance across batches of fish, however, the mean concentration across batches was utilized to calculate daily dose. Nonetheless, these results suggest that future efforts should utilize a single large composite mixture of fish to decrease dose variance.

Diet consisted of the NSRA-OCP fish sausages and control fish as a ration of 40% sausage and 60% control fish (silversides and capelin). Food consumption was recorded daily and is summarized in Figure 1, which demonstrates consistent mass consumption across time throughout this phase. However, the ratio of fish as sausages to control fish varied across days (Figure 2). Indeed, birds often times preferred the control fish to fish sausages, therefore, sausages were normally offered first, followed by control fish either after the sausages were consumed or during late afternoon. This technique resulted in significant variance in the daily ratio which indicated that future efforts should include control fish in sausage form or as a sausage containing a mixture of NSRA-OCP fish and control fish at the appropriate ratio might be better for future phases of study. Nonetheless, OCP

doses as µg//day consumed were more consistent across time than would be assumed from these ratio results (Figure 4). Although the results in Figure 4 suggests that OCPs were consumed at a reasonably consistent rate across time using this method, data are presented on a log basis and food consumption did vary with time, with significant decreases in food intake at later dates. Note both the reduced food intake and therefore reduced OCP intake during the later weeks (weeks 9-11) that were likely the normal seasonal decline in food intake in great egrets.

Every two weeks, birds were weighed and measured to monitor growth and assess general condition and health (Figure 5), and whole blood was collected to assess OCP concentrations and exposure (Figure 6). At the end of the experiment, birds were necropsied and whole blood, brain, liver, abdominal fat, feathers, and carcass remainders were collected for OCP analyses (Figures 7 and 8). The growth results, summarized in figure 5, indicate the maturation of hatchlings through fledgling and juvenile stages prior to the initiation of the OCP feeding trial. Body weights remained relatively constant through the feeding trial and therefore general condition and health was assessed as normal throughout this experiment.

Blood OCPs were monitored to potentially assess dietary exposures to OCPs as a function of time or exposure duration. However, blood concentrations were normally below or near detection limits and these analyses were limited. Blood OCP and lipid levels are summarized in Figure 6. Results indicate consistent concentrations of lipid, DDE and toxaphene in whole blood during the OCP feeding trial.

Tissue lipids and OCPs are summarized in Figures 7 and 8 respectively. Tissues (blood, brain, liver, abdominal fat, feathers and carcass remainder) were each analyzed for OCP concentrations following 56 and 74 days of dietary exposure. Results are limited in this report to the primary OCPs found at the NSRA: p,p' DDE, p,p' DDD, dieldrin, toxaphene, *alpha*-chlordane and *gamma*-chlordane, however other OCPs were routinely detected at lower concentrations and these data are included in the appendices. Results demonstrate significant tissue differences as expected, which indicates differential portioning of OCPs across tissues. Results suggest a tissue saturation for p,p' DDE, p,p' DDD, and *alpha*-chlordane as tissue levels did not differ between days 56 and 74 of exposure. However, significant increases in tissue levels were noted for dieldrin, toxaphene, and *gamma*-chlordane at day 74 as compared to day 56 of exposure.

Results for phase I indicate that the husbandry and experimental methods developed for this study are viable for dosing wading birds and for maintaining health. Sausage dosing resulted in significant exposures to the weathered OCPs routinely detected at the NSRA (p,p' DDE, p,p' DDD, dieldrin, toxaphene, *alpha*-chlordane and *gamma*-chlordane). However, health did not appear to be adversely affected by these OCP exposures and there were no signs of OCP toxicosis in egrets fed at a rate of 40% of their diet consisting of NSRA-OCP exposed fish. Blood concentrations of OCPs were not useful in assessing bioaccumulation nor the rates of accumulation. Future phases should include a sausage only diet, and higher doses of NSRA fish to evaluate the potential for NSRA derived OCP toxicosis in wading birds

Phase II: 2003

Eighteen great egret nestlings were collected from South Florida Water Conservation Area #3 on 4/22/2003 (birds # 1-18). Birds from this collection had several medical problems, including severe parasite loads and metabolic bone disease that necessitated that they not be utilized for this experimental trial and were therefore euthanized. An additional 15 great egret nestlings were collected on 4/28/2003 from a colony located at Gatorland alligator farm. The original study design called for 24 egrets, divided into four OCP dietary treatments (control, low, medium, and high OCPs) of six birds each, with an extra six birds collected to replace any possible losses. However, because of the much higher than expected mortality of birds from the south Florida collections, the study had to be re-designed to include only the surviving birds (n = 10) from Gatorland. Three birds were not dosed and tissues were collected for background contaminant analyses either prior to the start of OCP dosing (n=1) or at the conclusion of dosing (n=2), whereas the remaining seven birds were assigned to a dietary dosing for 8 weeks with NSRA-OCP-contaminated fish. However, one egret sustained significant wing and leg injuries at Week 3 and was euthanized; three egrets exhibited signs of poor health or potential toxicosis by 7 weeks and were euthanized (symptoms included lethargy, decreased food intake, tremors, inability to perch and poor mobility), and the remaining three egrets were euthanized at 8 weeks as per the experimental design.

Body measurements were recorded for all birds immediately following collection to estimate age and condition (Table 9). All birds were fed twice a day using a control diet of fish sausage which did not contain significant OCP residues (Table 11). One bird prior to OCP dosing and two additional birds following 8 weeks on a control fish diet were euthanized and samples collected for baseline OCP contaminant analyses (Table 10).

Diet consisted of 100% NSRA-OCP fish sausages which were produced in multiple batches and each analyzed for OCP concentrations (mean concentrations of approximately: 9,426 µg/kg p,p' DDE, 2,127 µg/kg p,p'DDD, 2,462 µg/kg dieldrin, 31,524 µg/kg toxaphene, 801 µg/kg *alpha*-chlordane, and 325 µg/kg *gamma*-chlordane). Food consumption was recorded daily and is summarized in Figure 9. OCP doses as µg/day consumed were consistent across time (Figure 9). However, note both the reduced food intake and therefore reduced OCP intake during the later weeks (weeks 7-8) that were likely the normal seasonal decline in food intake in great egrets as described in phase I.

Growth of egrets was determined by weighing and measuring (bill and tarsometatarsus length) each bird at a frequency of once a week until two months prior to the start of the dosing experiment (all measurements) and at the end of the dosing study (body weight only). At the end of the experiment, birds were necropsied and whole blood collected for blood chemistry analyses and hematological parameters (Tables 19 and 20 respectively). Brain, liver, abdominal fat, and the carcass remainder were also collected for OCP analyses (see OCP results in Table 21 and Figures 10 and 11).

During 2003, blood chemistry and hematology were variable with time (Tables 19 and 16, respectively) and were utilized to assess general heath status and any potential OCP toxicosis. At week 7, there was a significant increase in several blood chemistry parameters, including albumin (25% increase), calcium (40%), and phosphorous (56%). Only two parameters (ALK and CK) were decreased by weeks 7 and 8 (ALK 66 and 77% decline, respectively) and by week 8 (CK 48%) (Table 19). In relation to hematological parameters, although the total number of white blood cells remained the same over the course of the experiment, the percentage of lymphocytes increased by 43% whereas the percentage of heterophils declined by 50% (Table 20).

When conducting chemistry panels in birds, hepatic condition is usually assessed using three primary analyses: AST, CK, and albumin. Under normal circumstances, liver

enzymes (such as AST or aspartate aminotransferase) should not be found circulating in the blood stream. However, in cases of liver injury, liver cells (hepatocytes) lyse, "leaking" enzymes into the blood stream. However, AST can also be released by muscle cells (myocytes), thus an elevated AST is suggestive of either liver and/or muscle damage. Since CK (creatinine kinase) is only produced by myocytes, an elevated AST in the absence of an elevated CK suggests hepatocellular leakage and therefore liver disease. A fall in albumin concentrations is indicative of chronic liver disease. The opposite pattern (i.e. a decrease in ALK and CK and an increase in albumin) was observed in egrets dosed with OCPs for up to 8 weeks, suggesting that no liver damage was achieved. Although there was a significant increase in phosphorus plasma concentration at week 7, the almost 1:1 ratio of phosphorous to calcium, plus an absence of an increase in uric acid indicates absence of kidney damage.

An increase in relative lymphocyte counts (or lymphocytosis) could be indicative of an acute infectious disease; however an absence of an absolute increase in white blood cell numbers would indicate that this is an unlikely possibility. An increase in the relative percentage of lymphocytes was accompanied by an increase in the relative count of heterophils. Seasonal changes in the relative abundance of white blood cells similar to what was observed in this study (i.e. increase in lymphocytes and decrease in heterophils), has been reported in other avian species.

Tissue OCPs and lipids are summarized in Table 21 and Figure 10 respectively. Results are limited in this report to the primary OCPs found at the NSRA: p,p' DDE, p,p' DDD, dieldrin, toxaphene, *alpha*-chlordane and *gamma*-chlordane, however other OCPs were routinely detected at lower concentrations and these data are included in the appendices. Results demonstrate significant tissue differences as expected, which indicates differential portioning of OCPs across tissues as shown in phase I. OCP results indicated significant increases over time exposed. Egrets demonstrating potential signs of toxicosis at week 7 had similar OCP concentrations for tissues to those at week 8 for birds that did not exhibit toxicosis, however, variance within the week 7 birds was higher. One important difference was noted, that being the increased concentrations of OCPs in brain tissue for week 7 versus week 8 egrets. These data suggest a differential partitioning of OCPs in the birds exhibiting potential toxicosis and also suggests that the health effects observed may have been due to toxic levels of OCPs in the brain. Both phase I and phase II studies indicated significant dietary decrease over time,

which suggests potential dietary effects on the partitioning of OCPs across tissues. It is this differential partitioning and/or repartitioning of OCPs in response to decreased dietary intake that may have resulted in the toxicosis observed in phase II birds as well as a potential mechanism for what may have happened during the bird mortality event on the NSRA during 1998-99. Future studies should evaluate the effects of fasting on tissue uptake and the repartitioning of OCPs in great egrets.

Phase III: 2004

Thirty-three great egret nestlings were collected from North Central Florida during spring 2004. Three birds were euthanized following approximately 20 days of captivity (controls) and samples collected for baseline contaminant analyses. The remaining thirty birds were split into two groups and either fed/dosed with NSRA-OCP-contaminated fish sausage (n=22) or control fish (n=8) sausages for the remainder of this experimental trial. Food consumption was recorded daily. NSRA-OCP fed birds were sacrificed at three week intervals (weeks 3, 6, 9, 12, 15 and 18) through week 18 and at week 20 to assess bioaccumulation over time. At the conclusion of week 18, the remaining birds (n=10) were maintained for two additional weeks and sacrificed as follows: n=3 NSRA-OCP birds were maintained for two additional weeks on NSRA-OCP diets prior to sacrifice (Week 20 treated egrets); n=3 control birds maintained on control diets for two additional weeks prior to sacrifice (week 20 control egrets); and n=4 NSRA-OCP birds were starved (provided no food but water provided ad libitum) for two additional weeks prior to sacrifice (week 20 starved birds) to assess potential effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets. At the end of the experiment, birds were necropsied and whole blood, brain, liver, abdominal fat, subcutaneous fat and carcass remainders were collected for OCP analyses. Blood was also collected for the conduction of hematological and biochemistry panels.

Body measurements were recorded for all birds immediately following collection to estimate age and condition (see Table 15). All birds were fed twice a day using a control diet of fish which did not contain significant OCP residues (Table 17). Three birds were euthanized following approximately 20 days of captivity (controls) and samples collected for

baseline contaminant analyses (see Table 16) and tissue OCPs were background levels as expected. Birds were weighed and measured at the time of sacrifice and growth (body weight) is summarized in Figure 11). Diet consisted of 100% NSRA-OCP fish sausages which were produced in multiple batches and each analyzed for OCP concentrations (Table 18).

Birds were sacrificed every three weeks through week 18 and at week 20 to assess bioaccumulation as a function of time and results for blood chemistry, hematology and tissue OCPs are summarized in Tables 22 through 24. Blood chemistry and hematology were variable with time (Tables 22 and 23 respectively) and were utilized to assess general heath status.

Similarly to what was observed during 2003, blood chemistry and hematology were variable with time (Tables 22 and 23, respectively) and were utilized to assess general heath status. The most consistent changes included a decline in CK, phosphorous, and BUN/creatinine ratios starting on week 3 (range of declines of 39-89%, 42-70%, and of 42-70%, respectively); a decline in potassium during weeks 9 through 18 (28-37%); a decline in LDH during weeks 3 and 18 (42-68%); an increase in the concentration of plasma proteins starting at week 6 (19-41%); and an increase in creatinine, glucose, and cholesterol starting at week 3 (43-210%, 18-34%, and 38-88%, respectively) (Table 22). In terms of hematological changes, there was a significant decline in total number of white blood cells between weeks 3 and 12 (51-67%) which was due primarily to a decline in the number of lymphocytes (23-65%) (Table 23). Lymphocyte numbers were significantly increased between weeks 18 and 20 (47-68%), whereas heterophils were increased by week 3 (71%) and later decreased by weeks 18 and 20 (52-73%). In addition, there was an increase in the number of red blood cells and hematocrit at week 20 (29 and 23% increase, respectively).

As already discussed for the 2003 study, a lack of an increase in liver enzymes (i.e. AST, CK, and to some extent LDH) is indicative of functional livers. However, it is worth noting that during both years, there was a consistent decline in CK concentrations the cause of which remains unknown. On the other hand, the persistent high concentrations of creatinine could be indicative of kidney damage in the exposed birds. However, although hyperphosphatemias usually are another clinical sign of kidney failure, the opposite was

observed in the study birds. In addition, the abnormally high calcium to phosphorous ratios observed (these should approach 1 and were close to 3) could be indicative of some level of bone disease and/or nutritional disorder.

Increases in red blood cells and hematocrit are usually a reflection of dehydration, and thus represent just an “artificial” increase in red blood cell numbers due to hemoconcetration. Dehydrated animals also experience an increase in the concentration of plasma proteins, which was also observed in this study. The apparent immunosuppression observed throughout most of the study period contrasts to what was observed during 2003. Decline in lymphocyte numbers (lymphopenia) is a common stress response, and so are increases in glucose and cholesterol concentrations which were also observed in the birds studied. It appears though that by the end of the study (weeks 18 and 20) birds were recovering from this overall stress response, since white blood cell numbers had returned back to normal. Lymphocyte numbers were however above normal, and although morphological features of these cells were not provided by the laboratory, it is possible that most of these were immature stages of lymphocytes released to circulation to account for the overall low numbers. As already discussed, it is also possible that fluctuations in both heterophils and lymphocyte numbers over time is a normal seasonal phenomena in egrets, since it has been observed in other avian species.

Tissue OCP results for weeks 0 through 20 are summarized in Table 24. Results for tissue OCPs demonstrate significant bioaccumulation over time as expected. In general, saturation or peak levels were attained between weeks 9 and 18 of exposure depending on the analyte and tissue type. In general, levels of OCPs in blood were low, as expected, and were at maximum levels by week 9 of exposure. Carcass remainder) OCP concentrations had similar accumulation profiles as blood, regardless of analyte, with peak levels attained by week 9. Other tissues (brain, fat, liver) exhibited higher OCP concentrations as expected, with maximum levels attained between weeks 12 and 18.

At the conclusion of week 18 and the time dependent accumulation portion of the study, the remaining birds (n=10) were sacrificed as follows: n=3 NSRA-OCP birds were maintained for two additional weeks on NSRA-OCP diets prior to sacrifice (Week 20 treated egrets); n=3 control birds maintained on control diets for two additional weeks prior to sacrifice (week 20 control egrets); and n=4 NSRA-OCP birds were starved (provided no

food but water provided *ad libitum*) for two additional weeks prior to sacrifice (week 20 starved birds) to assess potential effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets. Blood chemistry was evaluated for each of these week 20 treatment groups and results are summarized in Tables 25 and 26. Blood chemistry and hematology were variable across week 20 treatments (Tables 25 and 26 respectively) but all birds were judged to be in good general health status.

After two weeks of starvation, some differences were noted for blood chemistry parameters for starved birds compared to controls (see Table 25). Changes included an increase in phosphorous (23%) and triglycerides (132%), as well as a decline in ALT (51%). An increase in the concentration of triglycerides makes sense, since birds were being starved and fat reserves were being mobilized to account for a lack of food intake. An increase in phosphorous could be an artifact of dehydration, although no significant increases were observed for hematocrit or plasma proteins. The cause for a decline in ALT remains unknown at this time.

In terms of hematological changes, there was an increase in the percentage of heterophils (50%) (Table 26). As already discussed, it appears that white blood cell numbers (especially lymphocytes and heterophils) vary seasonally in birds, and this could explain some of the changes observed.

Results for tissue OCPs across the 20 week treatments are summarized in Table 27. Results demonstrate significant bioaccumulation for all tissue as compared to controls as expected. Most importantly, significant repartitioning of OCPs across tissues occurred in response to fasting/starvation for 2 weeks. Carcass remainder OCP concentrations did not change in response to fasting, but the distribution of OCPs across tissue was altered significantly. In general, OCP concentrations were increased signficantly for all tissues tested (blood, brain, fat, and liver) as a result of fasting. This, in part, is likely due to a decrease in % lipid for the carcass remainder and liver in response to fasting as well. Results demonstrate a clear repartitioning of OCPs in egrets in response to fasting. This effect may, indeed, be a critical component of the mechanisms that resulted in avian mortalities on the NSRA during 1998-99.

Conclusions and Future Research Needs

During the fall of 1998 and winter of 1999, a significant bird mortality event occurred on the former agricultural properties on the north shore of Lake Apopka, an area now known as the North Shore Restoration Area (NSRA). The US Fish and Wildlife Service suggested that this mortality was linked to organochlorine pesticide (OCP) toxicosis. To evaluate thipotential link between OCPs and avian toxicosis, a series of three feeding studies were conducted using great egrets.

Great egrets were chosen for these feeding studies due to their high eco-relevance and their likely persistence in future NSRA ecosystems as a primary wading bird species. Nonetheless, much of the initial study (Phase I) was focused on the development and validation of husbandry and dosing procedures. These efforts demonstrated an ability to successfully raise and maintain great egret nestlings through adult ages under captive conditions. The procedures detailed in this report are likely among the first successful attempts to utilize great egrets as a laboratory model and the protocols produced should enable the future use of this model for other environmental assessments and study.

Dosing of great egrets for these feeding studies required the development of novel procedures that would enable adequate dietary intake comprised of specific fish sources as well as daily documentation and control of intake. All three phases of study utilized the production of ground fish sausages to accomplish these goals. The utilization of “sausages” enabled the production of a fish diet within the size and palatability appropriate for great egrets and the resultant approach should be a benchmark for the utilization of this and similar species in future captive wading bird assessments. Although labor intensive, these methods resulted in consistent food intake and adequate health maintenance through all life stages.

Great egrets were housed in outdoor chain linked enclosures developed and built for these studies. Enclosures had a floor space of 65 m^2 and a height of 128 cm and were constructed on top of a concrete basin. Each enclosure had multiple access gates (three on each side) and were covered with a clear roof. Enclosures also had running water (depth of < 3 cm), multiple perches, and standing blocks above the water line for birds to rest upon

and for positioning food trays. These facilities proved to be adequate for the maintenance of great egrets under captive conditions and for the necessary feeding and husbandry needed within the limits of these experimental trials. Future efforts with great egrets of other wading bird species would likely be equally successful in these or similar facilities.

Studies were conducted in three phases during 2002 through 2004: Phase I - to determine the potential for bioaccumulation of weathered organochlorine pesticides (OCP) in great egrets (*Casmerodius albus*) fed diets consisting of primarily blue tilapia (*Oreochromis aurea*) exposed to OCP-contaminated soils/sediments from the NSRA and evaluate health and growth effects; Phase II – to determine the potential for toxicosis from *in situ* weathered OCPs in great egrets; and Phase III – to evaluate the effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets. These feeding studies were conducted during the spring and summer of three consecutive years (2002 to 2004).

During 2002 (Phase I), ten birds were fed a diet consisting of approximately 40% NSRA-OCP-contaminated tilapia. Five birds were euthanized at 9 weeks and five at 11 weeks to evaluate bioaccumulation of OCPs. Results demonstrated significant bioaccumulation of OCPs from dietary intake of NSRA-tilapia through 18 weeks in great egrets. Saturation or equilibrium levels of OCPs varied with analyte and between experimental phases. Nonetheless, results clearly demonstrated a significant bioaccumulation of OCPs across tissues for great egrets fed a diet of NSRA-raised tilapia.

During 2003 (Phase 2), ten birds were dosed as follows: three controls and 7 treated (fed 100% NSRA-OCP-contaminated tilapia sausage for 8 weeks). Four treated egrets exhibited signs of poor health or potential toxicosis by 7 weeks and were euthanized (symptoms included lethargy, decreased food intake, tremors, inability to perch and poor mobility), and the remaining egrets were euthanized at 8 weeks as per the experimental design. These results demonstrated that a 100% diet of NSRA tilapia sausage was capable of a resultant toxicosis in some portion of the birds treated, which could, in part, explain the 1998-99 avian mortality event reported for the NSRA.

During 2004 (Phase III), thirty birds were split into two groups and either fed/dosed with 100% diet of NSRA-OCP-contaminated fish sausage (n=22) or control fish (n=8) for 18 to 20 weeks. NSRA-OCP fed birds were sacrificed at three week intervals (weeks 3, 6, 9, 12, 15 and 18) through week 18 to assess bioaccumulation over time. At the conclusion of

week 18, the remaining birds (n=10) were maintained for two additional weeks and sacrificed as follows: n=3 NSRA-OCP birds maintained as treated (Week 20 treated egrets); n=3 control birds maintained on control diets (week 20 control egrets); and n=4 NSRA-OCP birds which were starved (provided no food but water provided ad libitum; week 20 starved birds) to assess effects of fasting on tissue bioaccumulation and re-partitioning of OCPs in great egrets. These results indicated that seasonal and/or health derived reductions in dietary intake would repartition OCPs and that this mechanism may be a critical component in the toxicosis of OCPs in wading birds.

Future efforts will likely need to focus on natural exposures and wading birds on the NSRA sites. Restoration activities are ongoing and planned, which may produce wading bird habitats. The feeding trials summarized within this report indicate a significant risk of exposure for wading birds on the NSRA. However, the potential for adverse effects are less certain. Indeed, the feeding trials indicate that adverse effects are dependent upon the fraction of contaminated fish in the diet and are likely the product of interactions with additional stressors, such as reduced food-intake. Nonetheless, adequate monitoring of exposure and effects in wading birds across the NSRA will be necessary.

Tables

Table 1. Summary of experimental design/treatments for great egrets (n = 15) (Phase I study).

Bird #	Sex:	Day Euthanized:	Nest:	Treatment Group:	Date Euthanized:
1	M	56	1	A	10/28/2002
2	F	74	2	B	11/15/2002
3	F	-55	2	Control	7/8/2002
4	M	-90	3	Control	6/4/2002
5	M	56	4	A	10/28/2002
6	F	-90	4	Control	6/4/2002
7	M	74	5	B	11/15/2002
8	M	-90	6	Control	11/15/2002
9	M	56	7	A	10/28/2002
10	M	74	8	B	11/15/2002
11	F	74	9	B	11/15/2002
12	M	56	10	A	10/28/2002
13	M	56	11	A	10/28/2002
14	F	74	12	B	11/15/2002
15	M	-21	13	Control	8/12/2002

Table 2. Summary tissue collections for each experimental treatment for great egrets (Phase I study).

			Tissue Collected					
Exp. Day	**Date**	**Treatment Group:**	**Blood**	**Abdominal Fat**	**Brain**	**Liver**	**Feathers**	**Carcass Remainder**
-90	6/4/2002	Control	X	X	X	X	X	X
-55	7/8/2002	Control	X	X	X	X	X	X
-21	8/12/2002	Control	X	X	X	X	X	X
0								
8	9/10/2002	A	X					
15	9/17/2002	B	X					
22	9/24/2002	A	X					
29	10/1/2002	B	X					
36	10/8/2002	A	X					
43	10/15/2002	B	X					
50	10/22/2002	A	X					
56	10/28/2002	A and B	X	X	X	X	X	X
71	11/12/2002	B	X					
74	11/15/2002	B	X	X	X	X	X	X

Table 3. Summary of body measurements of great egret nestlings (n = 15) at time of collection from South Florida during the spring of 2002 (Phase I study).

Body Measurement	Average	Standard Deviation	Range
Body Weight (g)	254	82	111 - 367
Bill Length (mm)	40	8.2	29 – 51
Tarsometatarsus Length (mm)	49	10	33 – 64

Table 4. Mean ± SE of baseline lipid (%) and organochlorine pesticide concentrations (µg/kg wet weight) in "clean" fishes used to feed the birds during the course of Phase I (2002) study. Natural sheep and pig intestinal casings used to make the fish sausages were also submitted for chemical analyses. In parentheses is number of samples submitted (lipid analyses) and the number that had detectable concentrations (other variables). All other OCPs, including toxaphene, were below the Method Detection Limit (MDL). (-* = Below MDL.)

Analyte	Capelin	Silversides	Intestinal Casings
Lipids	5.48 ± 0.2 (5)	7.14 ± 0.9 (2)	1.67 ± 1.6 (2)
p,p'-DDD	0.79 ± 0.1 (4)	1.20 (1)	-*
p,p'-DDE	1.86 ± 0.2 (5)	5.55 ± 0.5 (2)	-*
Toxaphene	-*	-*	-*
Dieldrin	1.40 ± 0.1 (5)	-*	-*
alpha-Chlordane	3.16 ± 0.2 (5)	5.10 ± 0.8 (2)	-*
gamma-Chlordane	-*	-*	-*

Table 5. Mean ± SE of baseline lipid (%) and organochlorine pesticide concentrations (µg/kg, wet weight) in tissues of three great egret nestlings collected from South Florida, Water Conservation Area 3 in spring of 2002 (Phase I study). In parentheses number of birds that had detectable concentrations. All other OCPs, including toxaphene, were below the Method Detection Limit (MDL). (-* = Below MDL.)

Analyte	Whole Blood	Brain	Liver	Abdominal Fat
Lipids	0.45 ± 0.05 (3)	7.4 ± 0.4 (3)	4.7 ± 0.3 (3)	81 ± 8.2 (3)
p,p'-DDD	-*	33 (1)	-*	21 (1)
p,p'-DDE	-*	180 (1)	130 (1)	190 ± 50 (2)
Dieldrin	-*	470 (1)	25 ± 8.6 (3)	380 ± 40 (2)
Toxaphene	-*	-*	-*	-*
alpha -Chlordane	-*	-*	26 (1)	-*
gamma -Chlordane	-*	-*	-*	-*

Table 6. Organochlorine pesticide concentrations (µg/kg, wet weight) in blue tilapia sausages during Phase I (2002) study. Tilapia were "naturally" exposed to pesticides after stocking a clean population of fish into ponds constructed on the former Hooper Farm field. Tilapia were harvested after 2 months of bioaccumulation. Each collection event represents the composite of three independent samples. Overall average was used to calculate exposure rates.

Date of Collection	p,p'-DDE	p,p'-DDD	Dieldrin	Toxaphene	*alpha* - Chlordane	*gamma*- Chlordane
8/21/02	4,033	833	987	13,000	240	83
9/13/02	3,167	790	893	12,333	387	77
9/30/02	1,733	387	553	7,067	177	64
10/22/02	2,300	600	677	13,000	230	114
10/29/02	2,233	537	587	14,333	167	100
Overall Average	**2,693**	**629**	**739**	**11,947**	**240**	**88**

Table 7. Summary of experimental design/treatments for great egrets (n = 10) (Phase II study).

Bird #	Sex:	Week Euthanized:	Nest:	Treatment Group:	Date Euthanized:
19	M	8	2	Control	9/9/2003
20	F	8	5	Control	9/9/2003
21	M	7	7	OCP	8/26/2003
22	M	4	9	OCP	8/4/2003
23	F	8	9	OCP	9/2/2003
24	F	7	12	OCP	8/22/2003
26	F	8	12	OCP	9/2/2003
27	M	8	13	OCP	9/2/2003
28	M	7	14	OCP	8/25/2003
30	M	-4	15	Control	6/5/2003

Table 8. Summary tissue collections for each experimental treatment for great egrets (Phase II study).

			Tissue Collected				
Exp. Week	**Date**	**Treatment Group:**	**Blood**	**Abdominal Fat**	**Brain**	**Liver**	**Carcass Remainder**
-4	6/4/2003	Control	X	X	X	X	X
0	7/7/2003	OCP & Control					
4	8/4/2003	OCP	X	X	X	X	X
7	9/17/2003	OCP	X	X	X	X	X
8	9/24/2003	OCP & Control	X	X	X	X	X

Table 9. Summary of body measurements of great egret nestlings (n = 30) at time of collection from South Florida and Gatorland during the spring of 2003 (Phase II study).

Body Measurement	Average	Standard Deviation	Range
Body Weight (g)	384	38	220 - 750
Bill Length (mm)	59	4.4	42 - 100
Tarsometatarsus Length (mm)	70	4.1	47 - 105

Table 10. Mean ± SE of baseline lipid (%) and organochlorine pesticide concentrations (µg/kg, wet weight) in tissues of three control great egrets collected from Gatorland Aligator Farm in spring of 2003 (Phase II study). Data are from birds 19 and 20 which served as controls and were sacrificed at the end of the study (9/9/03), and bird 30 which was sacrificed on 06/05/03 prior to the start of dosing. In parentheses number of birds that had detectable concentrations. (-*=below MDL)

Analyte	Brain	Liver	Abdominal Fat	Carcass Remainder
Lipids	5.7 ± 0.5 (3)	2.6 ± 0.3 (3)	91 ± 1.6 (2)	9.9 ± 4.5 (3)
p,p'-DDD	0.45 ± 0.1 (3)	-*	8.0 (1)	2.5 ± 0.6 (3)
p,p'-DDE	24 ± 11 (3)	18 ± 12 (3)	280 ± 10 (2)	39 ± 16 (3)
Dieldrin	9.0 (1)	4.7 ± 1.6 (3)	59 ± 2.0 (2)	8.3 ± 3.8 (3)
Toxaphene	-*	180 (1)	880 ± 20 (2)	122 ± 41 (3)
alpha-chlordane	5.0 (1)	-*	4.6 ± 0.3 (2)	3.7 ± 1.4 (3)
gamma–Chlordane	-*	-*	-*	1.5 ± 0.1 (2)

Table 11. Mean ± SE of baseline lipid (%) and organochlorine pesticide concentrations (µg/kg, wet weight) in "clean" tilapia used to feed the controls and the dosed birds prior to start of dosing during Phase II (2003) study. In parentheses number of samples that had detectable concentrations. All other OCPs were below the Method Detection Limit (-*, MDL).

Analyte	Concentration
Lipids	3.86 ± 1.3 (2)
p,p'-DDE	7.80 ± 6.2 (2)
p,p'-DDD	-*
Dieldrin	2.29 ± 1.5 (2)
Toxaphene	140 (1)
Transnonachlor	4.70 (1)
alpha-Chlordane	2.30 ± 0.4 (2)
gamma-Chlordane	1.7 (1)

Table 12. Organochlorine pesticide concentrations (µg/kg, wet weight) in blue tilapia sausages during Phase II (2003) study. Tilapia were "naturally" exposed to pesticides after stocking a clean population of fish into ponds constructed on the former Hooper Farm field a former agricultural site.

Date of Collection	p,p'-DDE	p,p'-DDD	Dieldrin	Toxaphene	*alpha* - Chlordane	*gamma*- Chlordane
7/7/03	6,200	1,700	1,600	53,000	980	430
7/15/03	5,900	1,600	1,600	52,000	980	480
8/1/03	5,500	1,500	1,500	52,000	920	420
8/15/03	5,500	1,500	1,400	52,000	920	190
Overall Average	**5,775**	**1,575**	**1,525**	**52,250**	**950**	**380**

Table 13. Summary of experimental design/treatments for great egret nestlings (n = 33) (Phase III study). Bird #49 was scheduled to be a week 15 treatment (trt) bird, however, the bird was killed by a raccoon prior to that date and results are not included in data summary.

Bird #	Sex:	Week Euthanized:	Nest:	Sibling Group:	Date Euthanized:	Comments:
31	M	3	1	A	7/13/2004	trt
32	M	20	1	A	11/10/2004	control
33	-	-	1	-	-	control
34	M	20	2	B	11/10/2004	control
35	F	6	2	B	8/23/2004	trt
36	F	9	2	B	8/23/2004	trt
37	M	20	2	B	11/10/2004	trt
38	F	18	3	C	10/27/2004	trt
39	F	20	3	C	11/10/2004	trt
40	M	20	3	C	11/10/2004	starved
41	M	20	4	D	11/10/2004	starved
42	M	12	4	D	9/15/2004	trt
43	-	-	4	-	-	control
44	M	20	5	E	11/10/2004	control
45	-	-	5	-	-	control
46	-	-	5	-	-	control
47	M	-3	6	F	6/1/2004	trt
48	F	3	6	F	7/13/2004	trt
49	M	-	6	F		trt, Killed by raccoon
50	M	20	6	F	11/10/2004	starved
51	F	9	7	G	8/23/2004	trt
52	M	20	7	G	11/10/2004	starved
53	M	-3	7	G	5/28/2004	trt
54	M	20	8	H	11/10/2004	trt
55	-	-	8	-	-	control
56	M	18	8	H	10/27/2004	trt
57	M	12	8	H	9/15/2004	trt
58	F	20	9	I	11/10/2004	control
59	M	18	9	I	10/27/2004	trt
60	M	15	9	I	10/5/2004	trt
61	M	20	10	J	11/10/2004	control
62	F	6	10	J	8/23/2004	trt
63	M	-3	10	J	6/3/2004	trt

Table 14. Summary tissue collections for each experimental treatment for great egrets (Phase III study).

				Tissue Collected				
Exp. Week	**Date**	**N**	**Treatment Group:**	**Blood**	**Abdominal Fat**	**Brain**	**Liver**	**Carcass Remainder**
-3	5/28-6/3/2004	3	Control	X	X	X	X	X
0	6/20/2004	0	OCP & Control					
3	8/4/2004	2	OCP	X	X	X	X	X
6	9/17/2004	2	OCP	X	X	X	X	X
9	9/24/2004	2	OCP	X	X	X	X	X
12	9/15/2004	2	OCP	X	X	X	X	X
15	10/5/2004	1	OCP	X	X	X	X	X
18	10/27/2004	3	OCP	X	X	X	X	X
20	11/10/2004	3	OCP	X	X	X	X	X
20	11/10/2004	3	Control	X	X	X	X	X
20	11/10/2004	4	OCP-starved	X	X	X	X	X

Table 15. Summary of body measurements of great egret nestlings (n = 30) at time of collection from north Central Florida during the spring of 2004 (Phase III study).

Body Measurement	Average	Standard Deviation	Range
Body Weight (g)	749	124.5	450 - 970
Bill Length (mm)	76	6.9	56 – 88
Tarsometatarsus Length (mm)	121	13.2	80 – 142

Table 16. Mean ± SE of baseline lipid (%) and organochlorine pesticide concentrations (µg/kg, wet weight) in tissues of three great egret nestlings collected from North Central, in spring of 2004 (Phase III study). In parentheses number of birds that had detectable concentrations.

Analyte	Whole Blood	Brain	Liver	Abdominal Fat	Carcass Remainder
Lipids	0.48 (1)	6.8 ± 0.7 (3)	4.9 ± 0.6 (3)	73 ± 9.4 (3)	8.5± 0.7 (3)
p,p'-DDD	2.9 (1)	3.2 ± 0.3 (3)	1.2 ± 0.3 (3)	22 ± 5.5 (3)	4.3 ± 0.8 (3)
p,p'-DDE	7.2 (1)	5.2 ± 0.8 (3)	13.7 ± 0.9 (3)	380 ± 69 (3)	46 ± 9.5 (3)
Dieldrin	3.0 (1)	3.4 ± 0.6 (3)	8.8 ± 1.1 (3)	133 ± 23 (3)	14.6 ± 0.8 (3)
Toxaphene	130 (1)	147 ± 21 (3)	80 ± 11 (3)	2433 ± 321 (3)	200 ± 31 (3)
alpha-Chlordane	1.2 (1)	1.33 ± 0.3 (3)	0.7 ± 0.2 (3)	12.9 ± 1.6 (3)	7.5 ± 1.2 (3)
gamma-Chlordane	4.6 (1)	5.1 ± 0.9 (3)	1.3 ± 0.4 (3)	10.1 ± 0.9 (3)	1.3 ± 0.5 (3)

Table 17. Mean ± SE of baseline lipid (%) and organochlorine pesticide concentrations (µg/kg wet weight) in "clean" fishes used to feed the birds during the course of Phase III (2004) study. Natural sheep intestinal casings used to make the fish sausages were also submitted for chemical analyses. In parentheses number of samples that had detectable concentrations. All other OCPs were below the Method Detection Limit (MDL).

Analyte	Tilapia	Intestinal Casings
Lipids	4.07 ± 1.2	1.58 ± 1.7
p,p'-DDD	5.63 ± 5.6 (3)	-*
p,p'-DDE	5.17± 2.3 (3)	1.21 (1)
Dieldrin	1.63 ± 0.7 (3)	-*
Toxaphene	118 (1)	-*
alpha-Chlordane	1.56 ± 0.3 (2)	-*
gamma-Chlordane	-*	-*

- = Below MDL.

Table 18. Organochlorine pesticide concentrations (µg/kg, wet weight) in blue tilapia sausages during Phase III (2004) study. Tilapia were "naturally" exposed to pesticides after stocking a clean population of fish into ponds constructed at the Hooper Site a formerly agricultural site on the NSRA.

Date of Collection	p,p'-DDE	p,p'-DDD	Dieldrin	Toxaphene	*alpha*-Chlordane	*gamma*-Chlordane	Lipids Percent
6/22/04	7800	1200	380	10,000	330	200	2.68
7/14/04	6000	810	310	9,000	270	180	2.25
7/29/04	7300	1200	380	9,900	330	230	2.59
8/8/04	5000	760	430	10,000	320	170	2.62
8/12/04	7900	1100	400	11,000	360	200	2.82
8/16/04	7300	1100	370	10,000	330	180	2.74
8/22/04	7300	1300	350	9,600	320	240	2.59
8/28/04	7500	1400	370	10,000	350	250	2.62
9/4/04	7000	920	340	10,000	310	150	2.40
9/19/04	7000	1100	360	9,700	330	160	2.89
10/1/04	6100	850	310	8,400	280	190	3.00
10/5/04	6400	1300	310	8,800	280	200	3.38
10/14/04	2900	590	350	7,200	310	120	2.74
10/20/04	3200	660	210	7,900	350	170	2.84
10/29/04	2600	590	330	7,400	300	140	2.80
Overall Average	**6,087**	**992**	**347**	**9,260**	**318**	**185**	**2.54**

Table 19. Mean ± SE plasma biochemistry for great egrets during the course of Phase II study (2003). Three birds were not dosed (Controls), while seven birds were assigned to a dietary dosing for 8 weeks with NSRA-OCP fish. One egret was euthanized at week 3; three egrets exhibited signs of potential toxicosis by 7 weeks and were euthanized, and three egrets were euthanized at 8 weeks. Means with different superscript letters (within rows) are significantly different from each other (ANOVA, week 3 not included in analysis).

Parameter	Control	Week 3	Week 7 (potential toxicosis)	Week 8
Anion Gap	37 ± 2.5	30	46 ± 11	35 ± 1.3
Albumin (g/dL)	1.6 ± 0.07[a]	1.4	2.0 ± 0.03[b]	1.6 ± 0.06[a]
ALK (U/L)	232 ± 33[a]	135	53 ± 12[b]	79 ± 13[b]
AST (U/L)	111 ± 10	82	105 ± 14	83 ± 7.9
Calcium (mg/dL)	8.2 ± 0.23[a]	9.4	11.4 ± 0.74[b]	-
Carbon Dioxide (mEq/L)	13 ± 1.5	12	10 ± 3.5	15 ± 0.33
Chloride (mEq/L)	110 ± 0.75	113	112 ± 1.9	112 ± 0.88
Creatinine Kinase (U/L)	1791 ± 178[a]	1373	1626 ± 222[ab]	924 ± 68[b]
Globulin (g/dL)	2.4 ± 0.09	2.1	2.4 ± 0.35	2.2 ± 0.17
Glucose (mg/dL)	338 ± 11	381	309 ± 18	323 ± 18
Magnesium (mEq/L)	1.9 ± 0.19	1.6	2.7 ± 0.38	-
Phosphorus (mg/dL)	6.0 ± 0.30[a]	4.5	9.4 ± 0.49[b]	5.6 ± 0.96[a]
Plasma Proteins (g/dL)	3.9 ± 0.12	3.5	4.4 ± 0.38	3.8 ± 0.21
Potassium (mEq/L)	4.8 ± 0.42	3.4	6.5 ± 0.84	4.6 ± 0.41
Sodium (mEq/L)	155 ± 1.08	152	161 ± 6.2	157 ± 0.58
Uric Acid (mg/dl)	7.9 ± 0.73	3.2	7.7 ± 2.2	8.2 ± 1.7

Table 20. Mean ± SE hematological parameters for great egrets during the course of Phase II study (2003). Three birds were not dosed (Controls), while seven birds were assigned to a dietary dosing for 8 weeks with NSRA-OCP fish. One egret was euthanized at week 3; three egrets exhibited signs of potential toxicosis by 7 weeks and were euthanized, and three egrets were euthanized at 8 weeks. Means with different superscript letters (within rows) are different from each other (ANOVA, week 3 not included in analysis).

Parameter	Control	Week 3	Week 7 (potential toxicosis)	Week 8
Hemoglobin (g/dL)	-	-	15 ± 0.35	14 ± 0.29
Packed Cell Volume (%)	46 ± 0.95	46	50 ± 0.67	48 ± 1.5
Red Blood Cells ($x10^6$/µL)	2.1 ± 0.13	-	2.2 ± 0.17	1.9 ± 0.05
White Blood Cells ($x10^6$/µL)	14 ± 2.6	29	10 ± 2.1	9.3 ± 2.8
MCV (fL)	-	-	219 ± 15	248 ± 2.9
MCH (pg)	-	-	62 ± 4.4	72 ± 1.1
MCHC (g/dL)	-	-	30 ± 1.4	29 ± 0.35
Heterophils (%)	46 ± 5.5 ^{ab}	51	65 ± 15 ^{a}	23 ± 2.1 ^{b}
Heterophils (number/ µL)	5,042 ± 1,094	15,000	6,188 ± 1,707	2,166 ± 809
Lymphocytes (%)	38 ± 5.4 ^{ab}	38	25 ± 12 ^{a}	67 ± 6.1 ^{b}
Lymphocytes (number/ µL)	4,257 ± 894	11,000	2,684 ± 1,709	5,867 ± 1,534
Monocytes (%)	11 ± 1.7	9	7 ± 1.7	6.3 ± 2.0
Monocytes (number/ µL)	921 ± 233	2,600	717 ± 291	667 ± 348
Eosinophils (%)	3.7 ± 0.94	1	4.0	4.5 ± 1.5
Eosinophils (number/ µL)	389 ± 173	290	510	600 ± 200
Basophils (%)	1.0 ± 0	1	2.0 ± 0	2.0
Basophils (number/ µL)	97 ± 37	290	235 ± 15	400

Table 21. Mean ± SE tissue OCPs (µg/kg wet weight) in great egrets during course of Phase II study (2003). Three birds were not dosed (Controls), while seven birds were assigned to dietary dosing for 8 weeks with NSRA-OCP fish. One egret was euthanized at week 3; three egrets exhibited signs of potential toxicosis by 7 weeks and euthanized, and three egrets were euthanized at 8 weeks. Means with different superscript letters (within rows) are significantly different from each other (ANOVA, week 3 not included in analysis).

Analyte	Tissue	Control	Week 3	Week 7 (potential toxicosis)	Week 8
p,p' DDD	**Abdominal Fat**	8 ± 1.6^{a}	27000	89000 ± 31314^{b}	49333 ± 8029^{b}
	Blood	-	-	895 ± 611^{a}	1100 ± 99^{a}
	Brain	0.45 ± 0.1^{a}	130	467 ± 71^{b}	283 ± 29^{c}
	Carcass Remainder	2.5 ± 0.6^{a}	5000	6700 ± 889^{b}	9167 ± 498^{b}
	Liver	-	420	873 ± 167^{a}	1453 ± 576^{a}
p,p' DDE	**Abdominal Fat**	280 ± 10^{a}	100000	370000 ± 151522^{b}	203333 ± 34467^{b}
	Blood	-	-	3500 ± 2424^{a}	4150 ± 251^{a}
	Brain	24 ± 11^{a}	560	2367 ± 436^{b}	970 ± 119^{c}
	Carcass Remainder	39 ± 16^{a}	1700	25000 ± 3273^{b}	35667 ± 2960^{b}
	Liver	18 ± 12^{a}	1600	3933 ± 709^{b}	6100 ± 2210^{b}
Dieldrin	**Abdominal Fat**	59 ± 2^{a}	26000	96500 ± 33832^{b}	56667 ± 10267^{b}
	Blood	-	-	960 ± 745^{a}	1150 ± 151^{b}
	Brain	9 ± 0^{a}	230	813 ± 168^{b}	380 ± 45^{c}
	Carcass Remainder	8.3 ± 4^{a}	5200	7367 ± 1145^{b}	10667 ± 674^{b}
	Liver	4.7 ± 1.6^{a}	390	1090 ± 208^{b}	1770 ± 584^{b}

Table 21. (contd)

Analyte	Tissue	Week 0	Week 3	Week 7 (potential toxicosis)	Week 8
Toxaphene	**Abdominal Fat**	880 ± 20[a]	76000	2100000 ± 505075[b]	1233333 ± 148036[b]
	Blood	-	18	26300 ± 18890[a]	31500 ± 2525[b]
	Brain	-	4500	15567 ± 3003[a]	7400 ± 467[a]
	Carcass Remainder	122 ± 41[a]	16000	183333± 27169[b]	240000 ± 5882[b]
	Liver	180[a]	20000	30000 ± 6934[a]	43667 ± 8736[a]
***Alpha*-Chlordane**	**Abdominal Fat**	5 ± 0.3[a]	12000	38500 ± 12625[b]	24000 ± 5225[b]
	Blood	-		335 ± 237[a]	140 ± 5[a]
	Brain	5[a]	89	257 ± 36[b]	140 ± 25[c]
	Carcass Remainder	4 ± 1[a]	2300	2800 ± 458[b]	4400 ± 500[b]
	Liver	-	210	397 ± 78[a]	700 ± 171[a]
***Gamma*-Chlordane**	**Abdominal Fat**	-	-	15650 ± 6412[a]	11000 ± 2347[a]
	Blood	-	-	163 ± 106[a]	215 ± 5[a]
	Brain	-	35	99 ± 24[a]	76 ± 11[a]
	Carcass Remainder	1.5 ± 0.1[a]	1100	1177 ± 277[b]	2000 ± 355[b]
	Liver	-	100	160 ± 39[a]	283 ± 92[a]

Table 22. Mean ± SE of hematological parameters in plasma of great egrets during the course of Phase III study (2004). Samples were collected at the start of the experiment from all birds (n=28); from two birds at weeks 3, 6, 9, and 12, from 3 birds at weeks 18,and 20 Means with different superscript letters are significantly different from each other (ANOVA).

Parameter	Week 0	Week 3	Week 6	Week 9	Week 12	Week 18	Week 20
AST (U/L)	157 ± 15.2[a]	123 ± 7.1[a]	67 ± 11.1[b]	73 ± 4.0[b]	277 ± 39.8[c]	88 ± 13.9[a,b]	94 ± 20.1[a,b]
Calcium (mg/dL)	9.3 ± 0.3[a]	9.0 ± 0.5[a]	9.9 ± 0.2[a]	9.4 ± 0.4[a]	10.0 ± 0.2[a]	9.8 ± 0.1[a]	10.0 ± 0.4[a]
Carbon Dioxide (mmol/L)	21.8 ± 0.4[a]	18.5 ± 1.3[a]	19.0 ± 0.7[a]	23.5 ± 1.7[a]	24.5 ± 1.4[a]	25.3 ± 2.2[a]	27.3 ± 1.7[a]
Creatinine Kinase (U/L)	5452 ± 513[a]	3320 ± 672[b]	714 ± 57[c]	1213 ± 187[d]	665 ± 59[c]	605 ± 61[c]	773 ± 235[c]
Glucose (mg/dL)	254 ± 4.6[a]	341 ± 16.1[b]	306 ± 54.6[a,b]	336 ± 22.2[b]	340 ± 9.1[b]	301 ± 8.8[b]	333 ± 19.1[b]
Phosphorus (mg/dL)	6.6 ± 0.1[a]	2.6 ± 0.1[b]	4.4 ± 0.6[c]	2.5 ± 0.1[b]	3.8 ± 0[c]	2.0 ± 0.3[b]	3.3 ± 0.6[d]
Plasma Proteins (g/dL)	3.2 ± 0.1[a]	3.1 ± 0.1[a]	4.1 ± 0.3[b]	3.8 ± 0.4[a,b]	4.5 ± 0.5[b]	4.5 ± 0.4[b]	4.1 ± 0.2[b]
Potassium (mmol/L)	4.1 ± 0.3[a]	3.1 ± 0.3[a,b]	3.0 ± 0.4[a,b]	2.75 ± 0.3[b]	2.95 ± 0.3[b]	2.6 ± 0.3[b]	3.8 ± 0.3[a,b]
Sodium (mmol/L)	147 ± 2[a]	144 ± 1[a]	138 ± 1[a]	137 ± 1[a]	142 ± 2[a]	143 ± 2[a]	138 ± 1[a]
Uric Acid (mg/dl)	11.2 ± 0.6[a,b]	9.0 ± 0.2[a,c]	12.9 ± 2.9[b]	7.5 ± 1.5[c,d]	9.4 ± 0.3[a,c]	7.3 ± 1.3[c,d]	4.2 ± 1.6[d]

Table 22 (continued).

Parameter	Week 0	Week 3	Week 6	Week 9	Week 12	Week 18	Week 20
Total Bilirubin	0.56 ± 0.03	-	-	-	-	-	-
Blood Urea Nitrogen (BUN; mg/dL)	4.2 ± 0.3[a]	3.0 ± 0.4[a]	3.0 ± 0.5[a]	3.5 ± 0.6[a]	3.5 ± 0.4[a]	3.7 ± 0.4[a]	3.0 ± 0.6[a]
Creatinine (mg/dL)	0.42 ± 0.02[a]	0.7 ± 0.0[b,c]	1.3 ± 0.4[c]	0.6 ± 0.0[b]	0.75 ± 0.04[b,c]	0.73 ± 0.05[b,c]	0.80 ± 0.08[b,c]
Bun/Creat Ratio	10.2 ± 0.5[a]	4.3 ± 0[b]	3.0 ± 1.3[b]	5.9 ± 0.9[b]	4.65 ± 0.4[b]	4.96 ± 0.7[b]	3.80 ± 0[b]
ALT (U/L)	35.2 ± 4.1[a]	13.5 ± 8.6[a]	46.0 ± 31.2[a]	8.5 ± 1.5[b]	27.5 ± 3.5[a]	28.7 ± 1.8[a]	23.0± 1.6[a]
Lactic Dehydrogenase (U/L)	983 ± 40[a]	478 ± 43[b]	345 ± 17[b,c]	566 ± 154[b]	318 ± 3[c]	362 ± 30[b,c]	472 ± 108[b]
Amylase (U/L)	719 ± 36[a]	689 ± 41[a]	849 ± 37[a]	736 ± 159[a]	980 ± 175[a]	788 ± 75[a]	659 ± 159[a]
Lipase (U/L)	246 ± 19[a]	137 ± 46[a]	236 ± 67[a]	162 ± 28[a]	262 ± 45[a]	390 ± 131[a]	282 ± 93[a]
GGT (U/L)	7 ± 0.3[a]	6 ± 0[a]	6 ± 0[a]	6 ± 0[a]	<5	<5	<5
Cholesterol (mg/dl)	147 ± 5.3[a]	234 ± 2.5[b]	203 ± 27.2[b]	276 ± 37.9[b]	258 ± 62.1[b]	274 ± 22.1[b]	266 ± 29.4[b]
Triglycerides (mg/dL)	42.4 ± 4.1[a,b]	51.0 ± 3.0[a,c]	42.5 ± 0.5[a,b]	38.0 ± 1.0[b]	44.5 ± 0.5[a]	45.0 ± 0[a]	58.3 ± 3.8[c]

Table 23. Mean ± SE of hematological parameters in plasma of great egrets during the course of Phase III study (2004). Samples were collected at the start of the experiment from all birds (n=28); from two birds at weeks 3, 6, 9, and 12, from 3 birds at weeks 18 and 20. Means with different superscript letters are significantly different from each other (ANOVA).

Parameter	Week 0	Week 3	Week 6	Week 9	Week 12	Week 18	Week 20
Red Blood Cells ($x10^6$/µL)	3.8 ± 0.5[a]	3.7 ± 0.5[a]	3.6 ± 0.4[a]	3.6 ± 0.6[a]	3.6 ± 0.5[a]	4.1± 0.6[a]	4.9 ± 0.7[b]
White Blood Cells ($x10^6$/µL)	14.8 ± 0.9a	7.2 ± 0.9[b]	5.9 ± 0.7[b]	4.9 ± 0.8[b]	5.4 ± 0.8[b]	10.3 ± 2.4[a,b]	15.9 ± 2.5[a]
Heterophils (%)	48 ± 2.3[a]	82 ± 1.1[b]	57 ± 17.1[a,b]	49 ± 9.1[a,b]	61 ± 21.1[a,b]	23 ± 1.5[c]	13 ± 1.5[d]
Lymphocytes (%)	51 ± 2.3[a]	18 ± 0.9[b]	28 ± 4.5[b,c,d]	27 ± 2.1[c]	39 ± 1.4[d]	75 ± 1.9[e]	86 ± 1.8[e]
Monocytes (%)	0.2 ± 0.08	0	1 ± 0.05	0.5± 0.01	0	0	0
Eosinophils (%)	1 ± 0.3	0	15 ± 11.6	24 ± 10.6	0	2 ± 0.6	0
Basophils (%)	0	0	0	0	0	0	0
Hematocrit (%)	48.8 ± 0.8[a]	50 ± 2.0[a]	50 ± 1.6[a]	48 ± 2.6[a]	50 ± 5.1[a]	54 ± 1.8[a,b]	60 ± 1.8[b]

Table 24. Mean ± SE tissue OCPs (µg/kg wet weight) in great egrets during the course of Phase III study (2004). Samples were initially from all birds (n=28); and from two birds at weeks 3, 6, 9, and 12, from 3 birds at weeks 18 and 20. Means with different superscript letters are significantly different from each other (ANOVA).

Analyte	Tissue	Week 0	Week 3	Week 6	Week 9	Week 12	Week 18	Week 20
p,p' DDD	**Abdominal Fat**	22 ± 12^{a}	8200 ± 2828^{b}	21500 ± 3536^{c}	29333 ± 7101^{c}	31000 ± 10101^{c,d}	49667 ± 5071^{d}	38000 ± 401^{d}
	Blood	3	20 ± 3^{a}	35 ± 0^{b}	75 ± 5^{c}	80 ± 5^{c}	76 ± 9^{c}	77 ± 12^{c}
	Brain	3 ± 1^{a}	41 ± 2^{b}	115 ± 5^{c}	145 ± 25^{c,d}	155 ± 25^{c,d}	210 ± 31^{d}	173 ± 39^{d}
	Carcass Remainder	4 ± 1^{a}	2150 ± 252^{b}	3100 ± 101^{c}	9433 ± 1010^{d}	10300 ± 1717^{d}	12333 ± 1224^{d}	9600 ± 1542^{d}
	Liver	1 ± 0^{a}	190 ± 20^{b}	215 ± 15^{b,c}	430 ± 134^{c,d}	680 ± 222^{d,e}	917 ± 226^{e}	830 ± 36^{e}
	Subcutaneous Fat	-	-	-	-	-	-	40000 ± 2941
p,p' DDE	**Abdominal Fat**	380 ± 10^{a}	65500 ± 22728^{b}	145000 ± 25254^{b}	230000 ± 31452^{c}	205000 ± 49742^{c}	380000 ± 32751^{d}	356667 ± 26525^{d}
	Blood	7	190 ± 30a	285 ± 5^{b}	445 ± 6c	400 ± 20^{c}	650 ± 109^{d}	423 ± 88^{c,d}
	Brain	5 ± 1^{a}	435 ± 25^{b}	735 ± 56^{c}	985 ± 5^{d}	1350 ± 51^{e}	1933 ± 238^{f}	1900 ± 204^{f}
	Carcass Remainder	46 ± 5^{a}	18500 ± 1515^{b}	21000 ± 1010^{b}	92000 ± 14974^{c}	82000 ± 10101^{c}	94667 ± 10931^{c}	58633 ± 27700^{c}
	Liver	14 ± 4^{a}	1600 ± 202^{b}	1950 ± 51^{b}	3166 ± 694$_{c}$	5900 ± 1819^{c,d}	6133 ± 494^{d}	6300 ± 663^{d}
	Subcutaneous Fat	-	-	-	-	-	-	356666 ± 26525

Table 24. (cont'd)

Analyte	Tissue	Week 0	Week 3	Week 6	Week 9	Week 12	Week 18	Week 20
Dieldrin	**Abdominal Fat**	133 ± 9[a]	6450 ± 859[b]	14500 ± 2526[c]	19000 ± 1010[c]	16000 ± 1010[c]	30000 ± 2696[d]	25667 ± 1797[d]
	Blood	3	18 ± 3[a]	38 ± 1[b]	72 ± 6[c]	70 ± 3[c]	85 ± 9[c]	81 ± 10[c]
	Brain	3 ± 1[a]	49 ± 5[b]	115 ± 5[c]	140 ± 10[c,d]	160 ± 10[d]	253 ± 7e	250 ± 5[e]
	Carcass Remainder	15 ± 2[a]	1200 ± 202[b]	1850 ± 51[c]	5450 ± 354[d]	7400 ± 909[d]	7600 ± 972[d]	5500 ± 866[d]
	Liver	9 ± 3[a]	165 ± 1[b]	180 ± 0[c]	290 ± 66[d]	455 ± 45[d]	737 ± 98[e]	640 ± 68[e]
	Subcutaneous Fat	-	-	-	-	-	-	28667 ± 2652
Toxaphene	**Abdominal Fat**	2300 ± 166[a]	110000 ± 0[b]	200000 ± 10101[c]	255000 ± 15152[c,d]	280000 ± 10101[d]	336667 ± 17971[e]	473333 ± 27799[f]
	Blood	130	240 ± 0[a]	385 ± 5[b]	630 ± 71[c]	610 ± 91[c]	873 ± 100[c]	827 ± 14[c]
	Brain	147 ± 42[a]	635 ± 25[b]	1150 ± 51[c]	1500 ± 0[d]	1900 ± 202[d]	2600 ± 177[e]	2400 ± 257[e]
	Carcass Remainder	200 ± 21[a]	21000 ± 0[b]	22500 ± 5556[b]	83000 ± 27274[c]	77500 ± 4546[c]	84333 ± 6968[c]	102667 ± 9509[c]
	Liver	80 ± 12[a]	2450 ± 354[b]	2900 ± 202[b]	4067 ± 353[c]	6350 ± 656d	10166 ± 2155[e]	10000 ± 513[e]
	Subcutaneous Fat	-	-	-	-	-	-	386667 ± 32397
***alpha*-Chlordane**	**Abdominal Fat**	13 ± 3[a]	2850 ± 959[b]	6950 ± 1464[c]	11500 ± 505[d]	9900 ± 2121[d]	15666 ± 1358[e]	15000 ± 1019[e]
	Blood	1	10 ± 2[a]	17 ± 0[b]	34 ± 3[c]	30 ± 2[c]	38 ± 5[c]	37 ± 5[c]
	Brain	1 ± 1[a]	24 ± 3[b]	75 ± 11[c]	74 ± 7[c]	76 ± 4[c]	137 ± 22[d]	103 ± 29[c,d]
	Carcass Remainder	8 ± 0[a]	740 ± 20[b]	1195 ± 308[b]	4150 ± 151[c]	3350 ± 454[c]	4100 ± 102[c]	3600 ± 713[c]
	Liver	1± 0[a]	81 ± 20[b]	105 ± 5[b]	166 ± 55[b]	300 ± 10[c]	427 ± 109[c]	370 ± 12c
	Subcutaneous Fat	-	-	-	-	-	-	15000 ± 1765

Table 24. (cont'd)

Analyte	Tissue	Week 0	Week 3	Week 6	Week 9	Week 12	Week 18	Week 20
***gamma*-Chlordane**	**Abdominal Fat**	10 ± 1[a]	1600 ± 100[b]	2650 ± 51[c]	5300 ± 303[d]	4450 ± 1161[d]	3153 ± 1904[c,d]	4633 ± 392[d]
	Blood	5	7 ± 1[a]	6 ± 1[a]	15 ± 1[b]	11 ± 5[a,b]	10 ± 2[a,b]	9 ± 1[a,b]
	Brain	5 ± 2[a]	7 ± 3[a]	25 ± 2[b]	35 ± 2[c]	41 ± 10c	27 ± 12[b,c]	27 ± 2[b,c]
	Carcass Remainder	1 ± 0[a]	275 ± 66[b]	495 ± 5[c]	1100 ± 1151[d]	1350 ± 51[d]	1121 ± 576[d]	983 ± 111[d]
	Liver	1 ± 0[a]	34 ± 1[b]	60 ± 1[c]	110 ± 40[c,d]	160 ± 30[d]	115 ± 63[c,d]	86 ± 4[c]
	Subcutaneous Fat	-	-	-	-	-	-	4400 ± 176
% lipid	**Abdominal Fat**	73 ± 9[a]	92 ± 1[b]	85 ± 2[a,b]	87 ± 1[b]	88 ± 4[b]	94 ± 1[b]	93 ± 4[b]
	Blood	0.5	0.6 ± .02[a]	0.5 ± 0.01[b]	0.6 ± 0.02[a]	0.6 ± 0.03[a]	0.5 ± 0.03[b]	0.7 ± 0.15[a,b]
	Brain	6.8 ± 0.2[a]	7.2 ± 0.2[a]	7.0 ± 0.1[a]	7.0 ± 0.3[a]	7.4 ± 0.2[a]	7.2 ± 0.2[a]	7.0 ± 0.3[a]
	Carcass Remainder	8.5 ± 1.4[a]	19.1 ± 0.6[b,c]	15.7 ± 1.1[b]	23.2 ± 1.2[c]	23.9 ± 2.3[c]	24.6 ± 1.3[c]	25.8 ± 4.0[c]
	Liver	4.0 ± 0.2[a]	4.6 ± 0.4[a,b]	3.7 ± 0.3[a]	3.6 ± 0.6[a,b]	4.3 ± 0.1[a,b]	5.4 ± 0.5[b]	13.9 ± 8.8[c]
	Subcutaneous Fat	-	-	-	-	-	-	89 ± 4

Table 25. Mean ± SE of hematological parameters in plasma of great egrets during the course of Phase III study (2004). Samples were collected from three birds at week 20 for control and OCP-fed birds, and for four birds at week 20 for starved birds (birds were starved for duration of week 18 through week 20). Means with different superscript letters are significantly different from each other (ANOVA).

Parameter	Week 20 (control	Week 20 (OCP-fed)	Week 20 (starved)
AST (U/L)	147 ± 23.2[a]	94 ± 20.1[a]	103 ± 2.5[a]
Calcium (mg/dL)	10.1 ± 0.4[a]	10.0 ± 0.4[a]	10.0 ± 0.3[a]
Carbon Dioxide (mmol/L)	26.8 ± 2.6[a]	27.3 ± 1.7[a]	20.3 ± 1.7[a]
Creatinine Kinase (U/L)	1921 ± 728[a]	773 ± 235[b]	723 ± 178[b]
Glucose (mg/dL)	315 ± 25.4[a]	333 ± 19.1[a]	303 ± 6.9[a]
Phosphorus (mg/dL)	3.4 ± 0.6[a]	3.3 ± 0.6[a]	4.2 ± 0.7[b]
Plasma Proteins (g/dL)	3.4 ± 0.1[a]	4.1 ± 0.2[b]	4.1 ± 0.3[b]
Potassium (mmol/L)	3.3 ± 0.4[a]	3.8 ± 0.3[b]	3.5 ± 0.3[a,b]
Sodium (mmol/L)	138 ± 2[a]	138 ± 1[a]	136 ± 1[a]
Uric Acid (mg/dl)	4.8 ± 0.7[a]	4.2 ± 1.6[a]	4.4 ± 0.5[a]

Table 25 (continued).

Parameter	Week 20 (control	Week 20 (OCP-fed)	Week 20 (starved)
Total Bilirubin	-	-	-
Blood Urea Nitrogen (BUN; mg/dL)	3.8 ± 0.4[a]	3.0 ± 0.6[a]	4.5 ± 0.7[a]
Creatinine (mg/dL)	0.90 ± 0.12[a]	0.80 ± 0.08[a]	1.28 ± 0.2[a]
Bun/Creat Ratio	4.18 ± 0.3[a]	3.80 ± 0[a]	3.65 ± 0.8[a]
ALT (U/L)	23.4 ± 0.6[a]	23.0± 1.6[a]	11.3 ± 2.6[b]
Lactic Dehydrogenase (U/L)	911 ± 226[a]	472 ± 108[b]	381 ± 64[b]
Amylase (U/L)	597 ± 78[a]	659 ± 159[a,b]	905 ± 168[b]
Lipase (U/L)	175 ± 43[a]	282 ± 93[a]	204 ± 28[a]
GGT (U/L)	<5	<5	<5
Cholesterol (mg/dl)	221 ± 17.8[a]	266 ± 29.4[a]	296 ± 35.4[a]
Triglycerides (mg/dL)	53.3 ± 3.0[a]	58.3 ± 3.8[a]	123.5 ± 6.7[b]

Table 26. Mean ± SE of hematological parameters in plasma of great egrets during the course of Phase III study (2004). Samples were collected from three birds each at week 20 for control and OCP-fed birds, and for four birds at week 20 for starved birds (birds were starved for duration of week 18 through week 20). Means with different superscript letters are significantly different from each other (ANOVA).

Parameter	Week 20 (control	Week 20 (OCP-fed)	Week 20 (starved)
Red Blood Cells ($x10^6$/µL)	3.8 ± 0.4^a	4.9 ± 0.7^a	4.5 ± 0.6^a
White Blood Cells ($x10^6$/µL)	22.8 ± 3.5^a	$15.9 \pm 2.5^{a,b}$	13.1 ± 0.5^b
Heterophils (%)	61 ± 11.8^a	13 ± 1.5^b	31 ± 7.9^c
Lymphocytes (%)	39 ± 11.8^a	86 ± 1.8^b	69 ± 8.1^b
Monocytes (%)	0	0	0.2± 0.01
Eosinophils (%)	0	0	0
Basophils (%)	0	0	0
Hematocrit (%)	54 ± 2.2^a	60 ± 1.8^a	57 ± 7.6^a

Table 27. Mean ± SE tissue OCPs (µg/kg wet weight) in great egrets during the course of Phase III study (2004). Samples were collected from three birds each at week 20 for control and OCP-fed birds, and for four birds at week 20 for starved birds (birds were starved for duration of week 18 through week 20). Means with different superscript letters are significantly different from each other (ANOVA).

Analyte	Tissue	Week 20 (control	Week 20 (OCP-fed)	Week 20 (starved)
p,p' DDD	**Abdominal Fat**	25 ± 6^{a}	38000 ± 401^{b}	85750 ± 9187^{c}
	Blood	2 ± 0^{a}	77 ± 12^{b}	193 ± 1^{c}
	Brain	2 ± 1^{a}	173 ± 39^{b}	330 ± 73^{c}
	Carcass Remainder	15 ± 6^{a}	9600 ± 1542^{b}	11575 ± 1277^{b}
	Liver	1 ± 0^{a}	830 ± 36^{b}	1875 ± 287^{c}
	Subcutaneous Fat	32 ± 7^{a}	40000 ± 2941^{b}	73500 ± 16322^{c}
p,p' DDE	**Abdominal Fat**	500 ± 40^{a}	356667 ± 26525^{b}	662500 ± 43661^{c}
	Blood	12 ± 2^{a}	423 ± 88^{b}	1460 ± 421^{c}
	Brain	19 ± 2^{a}	1900 ± 204^{b}	3450 ± 660^{c}
	Carcass Remainder	123 ± 27^{a}	58633 ± 27700^{b}	92000 ± 6570^{b}
	Liver	14 ± 2^{a}	6300 ± 663^{b}	15500 ± 2630^{c}
	Subcutaneous Fat	540 ± 10^{a}	356666 ± 26525^{b}	595000 ± 108742^{c}

Table 27. (contd)

Analyte	Tissue	Week 20 (control	Week 20 (OCP-fed)	Week 20 (starved)
Dieldrin	Abdominal Fat	41 ± 5[a]	25667 ± 1797[b]	42000 ± 5788[c]
	Blood	2 ± 0[a]	81 ± 10[b]	193 ± 42[c]
	Brain	2 ± 0[a]	250 ± 59[b]	445 ± 85[c]
	Carcass Remainder	12 ± 6[a]	5500 ± 866[b]	6850 ± 1069[b]
	Liver	2 ± 1[a]	640 ± 68[b]	1475 ± 225[c]
	Subcutaneous Fat	41 ± 6[a]	28667 ± 2652[b]	37750 ± 4905[c]
Toxaphene	Abdominal Fat	995 ± 106[a]	473333 ± 27799[b]	692500 ± 139007[c]
	Blood	73 ± 5[a]	827 ± 142[b]	1825 ± 386[c]
	Brain	105 ± 16[a]	2400 ± 257[b]	4125 ± 73[c]
	Carcass Remainder	270 ± 91[a]	102667 ± 9509[b]	116000 ± 18655[b]
	Liver	48 ± 5[a]	10000 ± 513[b]	19000 ± 2041[c]
	Subcutaneous Fat	980 ± 121[a]	386667 ± 32397[b]	860000 ± 95786[c]
alpha-Chlordane	Abdominal Fat	14 ± 3[a]	15000 ± 1019[b]	32500 ± 3069[c]
	Blood	1 ± 0[a]	37 ± 5[b]	85 ± 14[c]
	Brain	1 ± 0[a]	103 ± 29[b]	205 ± 39[c]
	Carcass Remainder	6 ± 2[a]	3600 ± 713[b]	4050 ± 233[b]
	Liver	0 ± 0[a]	370 ± 12[b]	750 ± 118[c]
	Subcutaneous Fat	15 ± 3[a]	15000 ± 1765[b]	26500± 6225[c]

Table 27. (contd)

Analyte	Tissue	Week 20 (control	Week 20 (OCP-fed)	Week 20 (starved)
gamma-Chlordane	Abdominal Fat	5 ± 0^{a}	4633 ± 392^{b}	7350 ± 1178^{c}
	Blood	3 ± 0^{a}	9 ± 1^{b}	22 ± 6^{c}
	Brain	3 ± 1a	27 ± 2^{b}	44 ± 10^{c}
	Carcass Remainder	1 ± 0^{a}	983 ± 111^{b}	923 ± 55^{b}
	Liver	2 ± 0^{a}	86 ± 4^{b}	183 ± 26^{c}
	Subcutaneous Fat	4 ± 0^{a}	4400 ± 176^{b}	6550 ± 1218^{c}
% lipid	Abdominal Fat	95 ± 2^{a}	$93 \pm 4^{a,b}$	89 ± 1^{b}
	Blood	0.2 ± 0.09^{a}	0.7 ± 0.15^{b}	0.6 ± 0.06^{b}
	Brain	6.9 ± 0.1^{a}	7.0 ± 0.3^{a}	7.1 ± 0.1^{a}
	Carcass Remainder	21.4 ± 0.8^{a}	25.8 ± 4.0^{a}	18.9 ± 0.9^{b}
	Liver	2.8 ± 1.3^{a}	13.9 ± 8.8^{b}	5.5 ± 0.3^{b}
	Subcutaneous Fat	90 ± 1^{a}	89 ± 4^{a}	84 ± 2^{a}

Figures

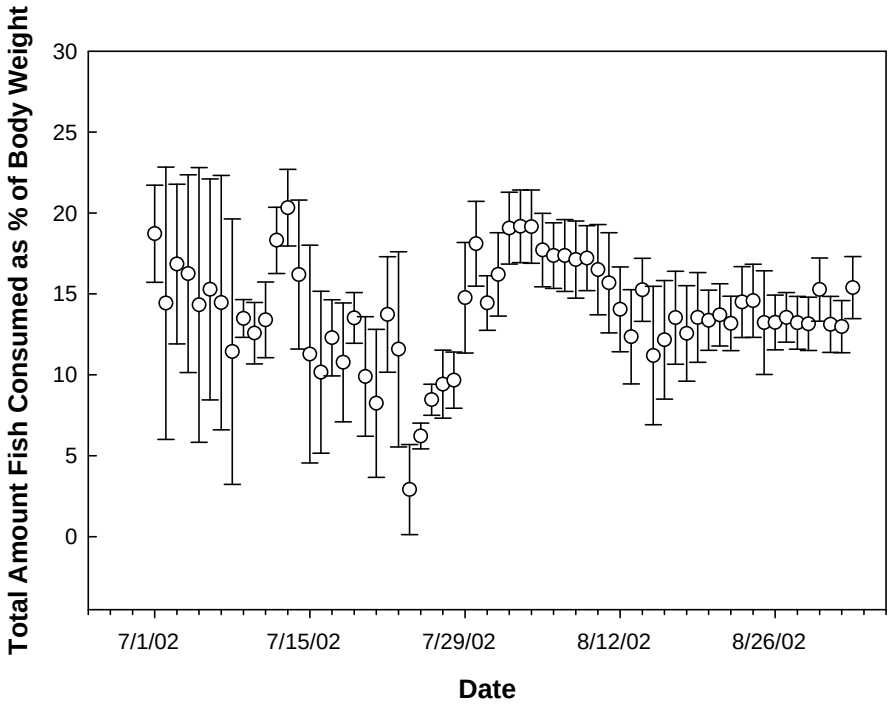


Figure 1. Mean ± standard deviation of total amount of fish consumed as percent of body weight for all ten egrets included in Phase I (2002) dosing experiment. Values shown represent acclimation period prior to initiation of OCP dosing. Throughout this time, egrets were trained on eating fish sausages.

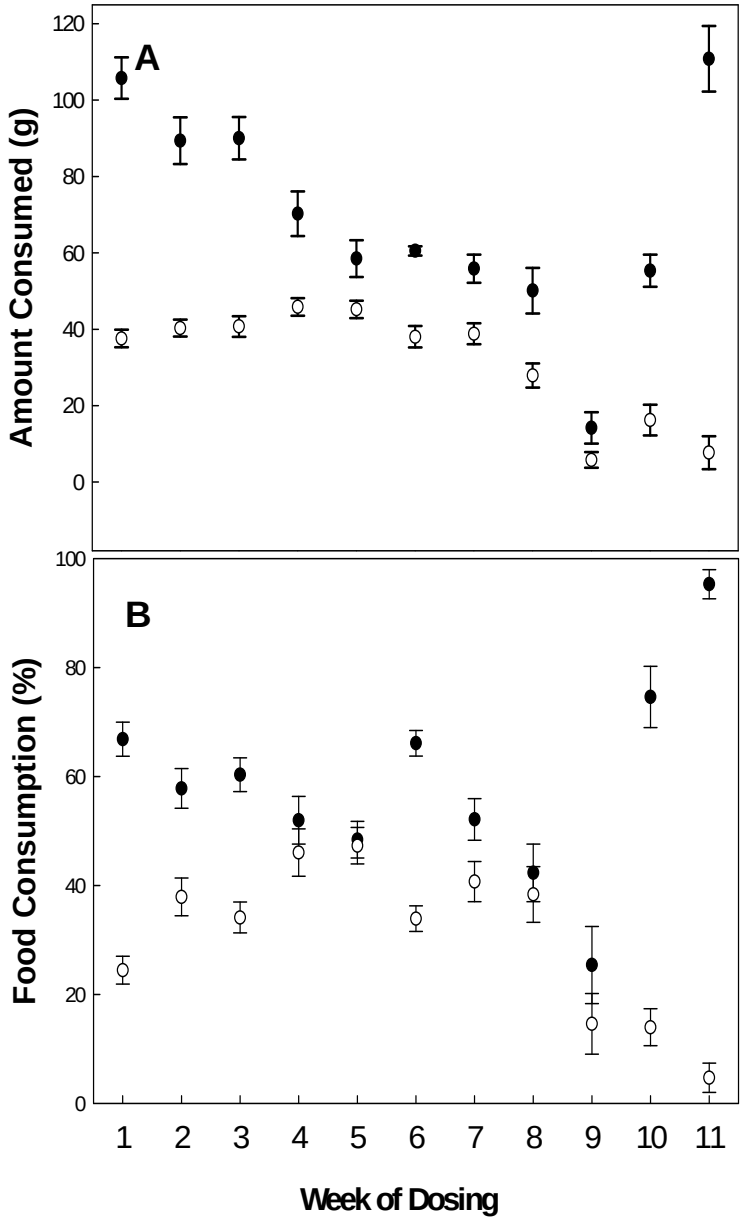


Figure 2. Summary of mean ± standard error of clean fish (closed circles) and OCP-contaminated sausage (open circles) consumed as grams/day (A) and as a percentage of total fish consumed/day (B) by week of dosing during Phase I (2002) study. Weeks 1 – 8, n = 10 egrets; weeks 9 – 11 = 5 egrets.

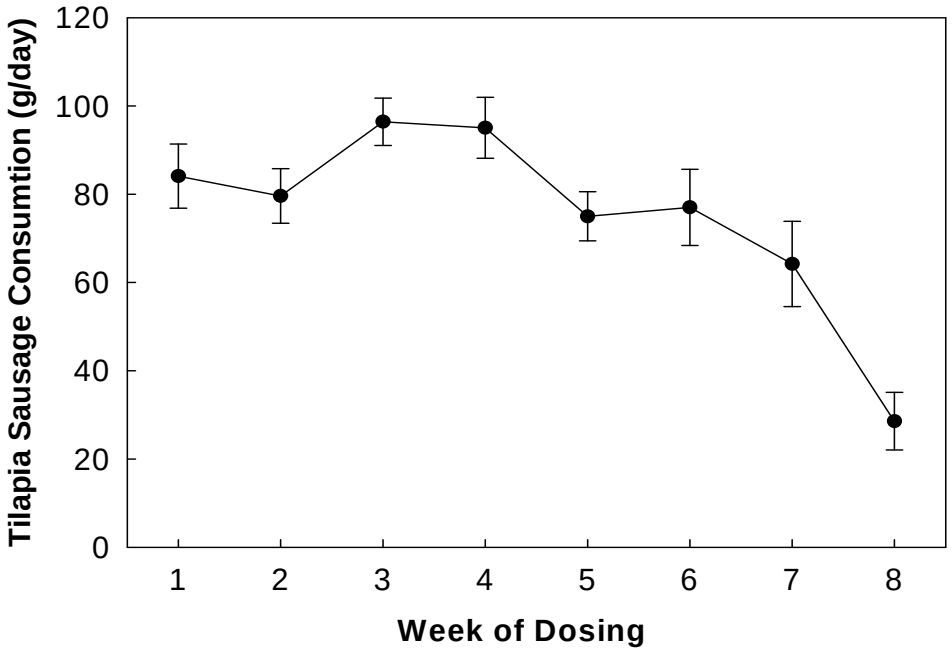


Figure 3. Summary of mean ± standard error of OCP-contaminated sausages consumed as grams/day by week of dosing during Phase II (2003) study. Weeks 1 – 4, n = 7 egrets; weeks 5 – 7 = 6 egrets; weeks 7 – 8 = 3 egrets.

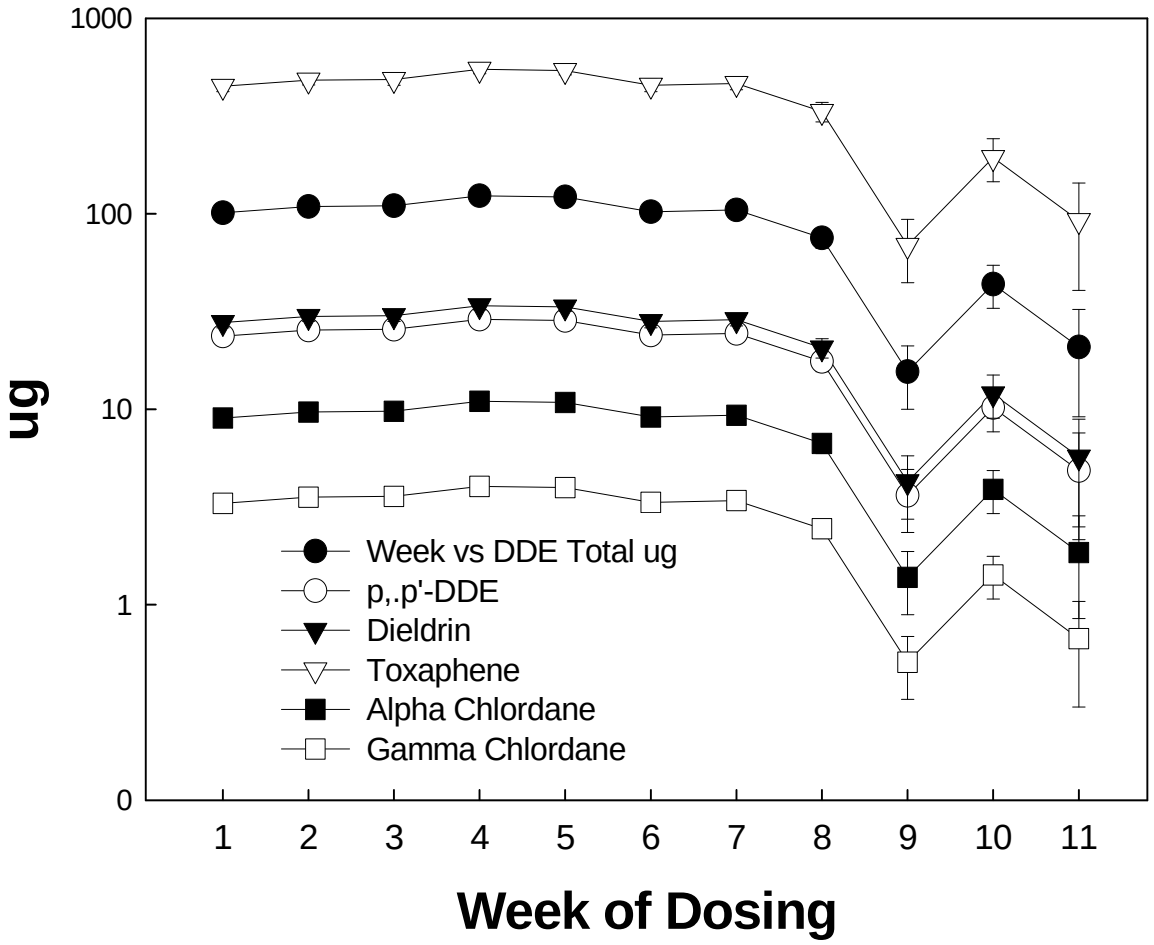


Figure 4. Summary of mean ± standard error of organochlorine pesticides consumed as total micrograms/day by week of dosing during Phase I (2002) study. Weeks 1 – 8, n = 10 egrets; weeks 9 – 11 = 5 egrets.

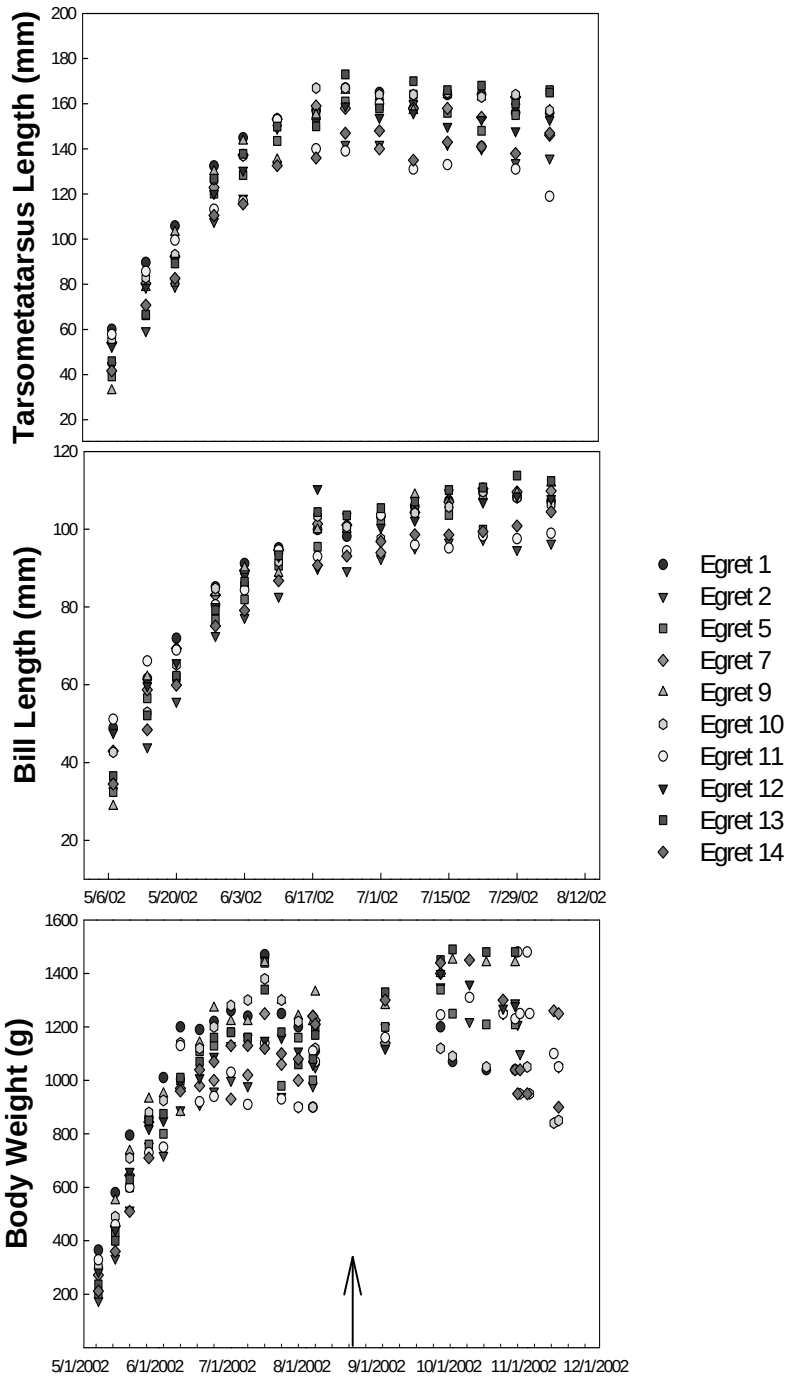


Figure 5. Summary of great egret growth from arrival to the laboratory (early May, all measurements) to end of OCP dosing study (early November, body weight only) for Phase I (2002) study. Arrow denotes the beginning of the OCP dosing study.

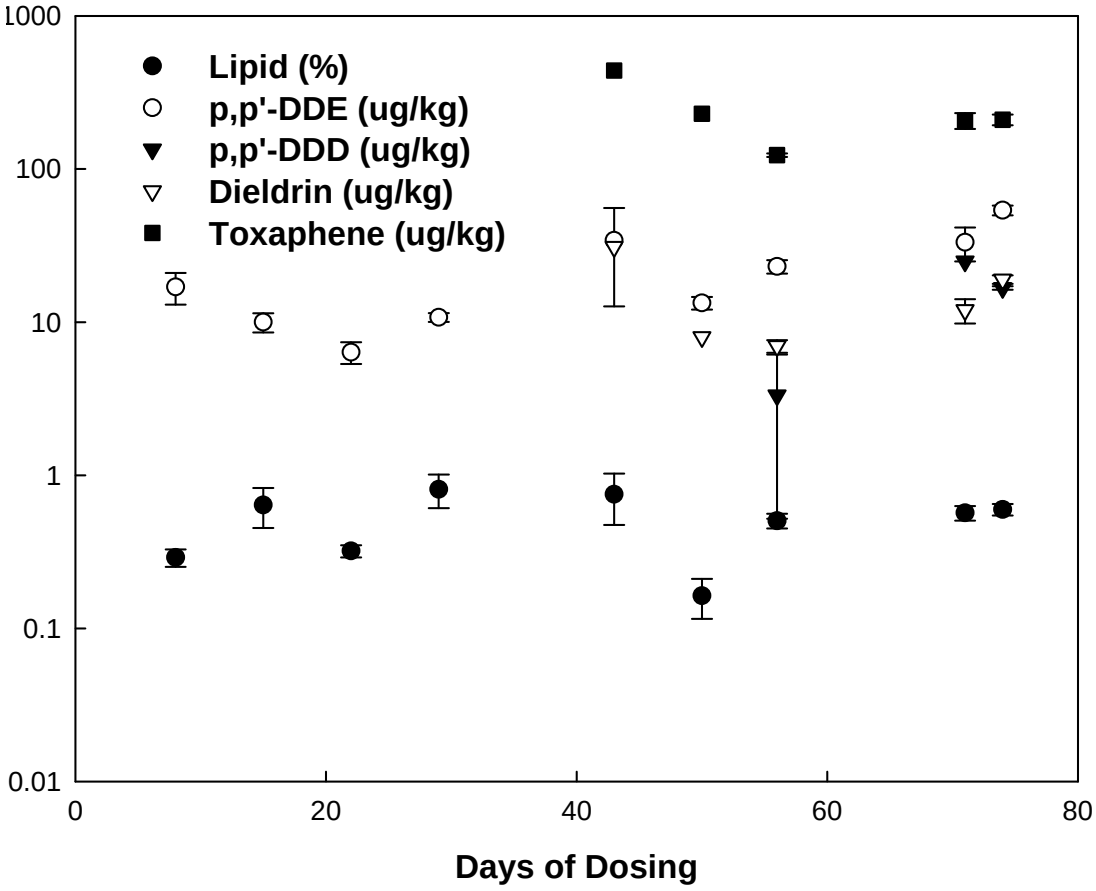


Figure 6. Mean ± standard error of changes in lipid (%) and organochlorine pesticides (µg/kg, wet weight) in whole blood in relation to days of dosing for Phase I (2002) study.

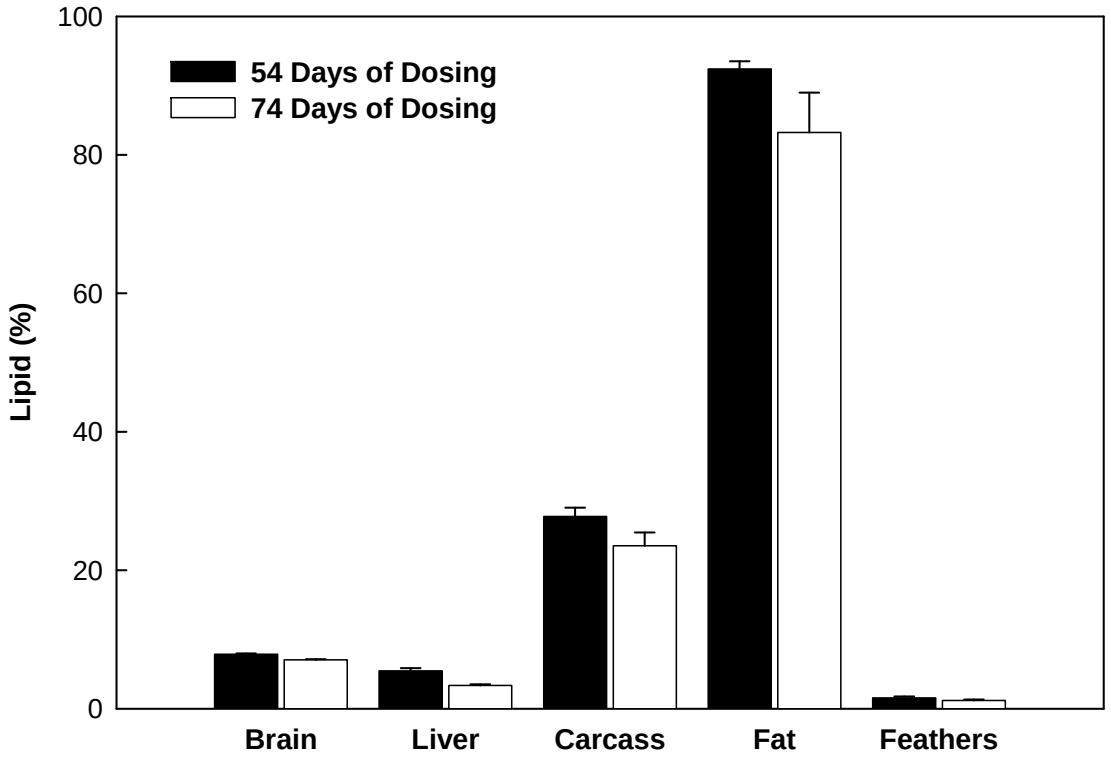


Figure 7. Mean ± standard error of lipid (%) in tissues of great egrets at the end of 56 or 74 days of organochlorine dosing for Phase I (2002) study. Each bar represents the average of tissues collected from five great egrets.

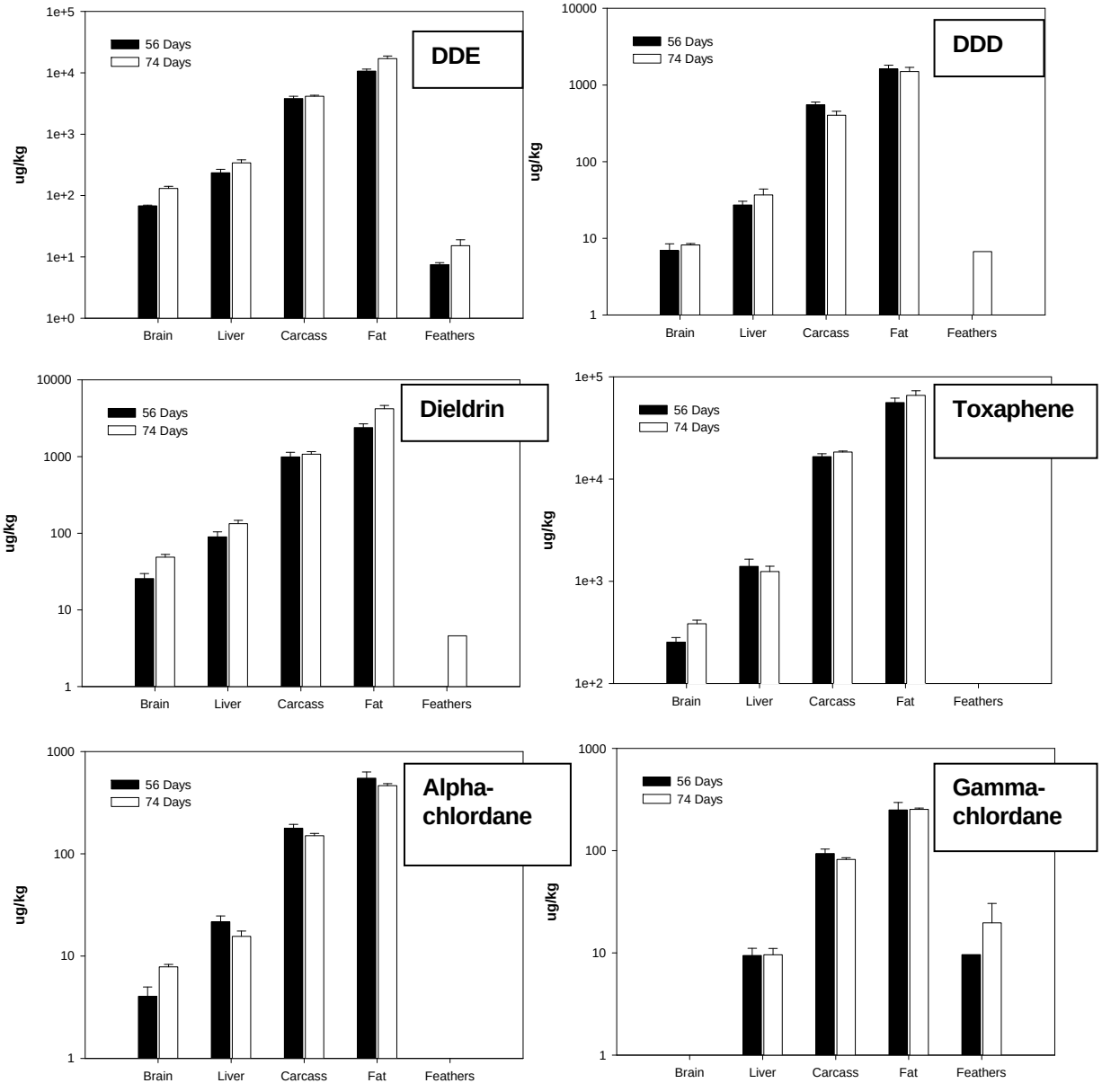


Figure 8. Mean ± standard error of main organochlorine pesticides (µg/kg), wet weight) in tissues of great egrets at the end of 56 or 74 days of dosing for Phase I (2002) study. Each bar represents the average of tissues collected from five great egrets.

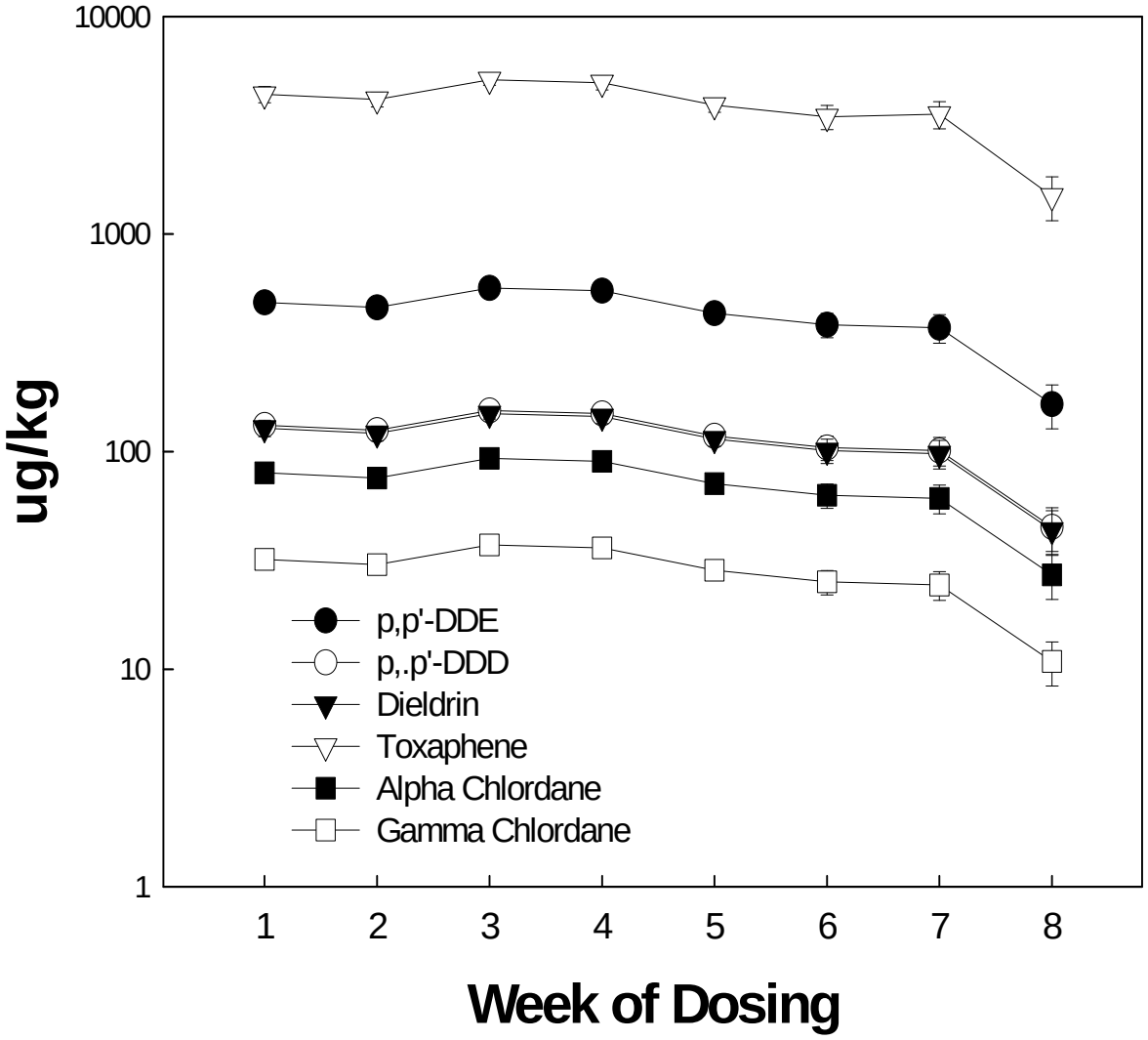


Figure 9. Summary of mean ± standard error of organochlorine pesticides consumed as total micrograms/day by week of dosing during Phase II (2003) study. Weeks 1 – 4, n = 7 egrets; weeks 5 – 7 = 6 egrets; weeks 7 – 8 = 3 egrets.

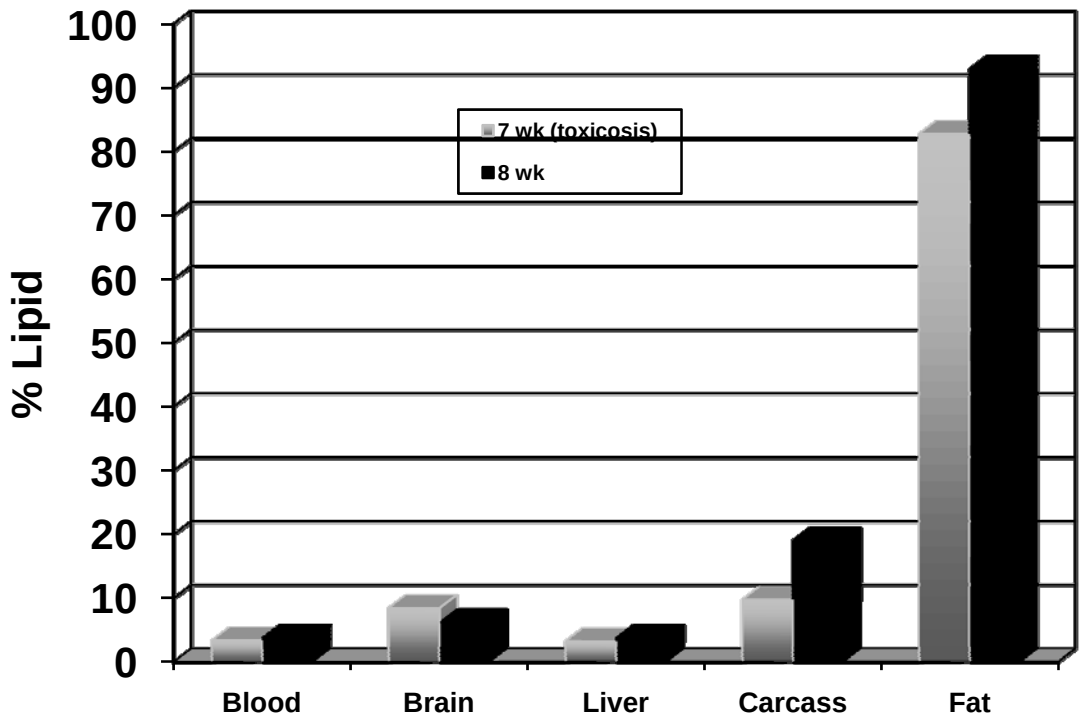


Figure 10. Mean lipid (%) in tissues of great egrets fed NSRA-OCP fish sausage at 7 weeks for birds that exhibited potential toxicosis and at 8 weeks for the remaining 3 birds; Phase II (2003) study. Significant differences were not noted except for reduced lipid in carcass for week 7 (toxicosis) birds.

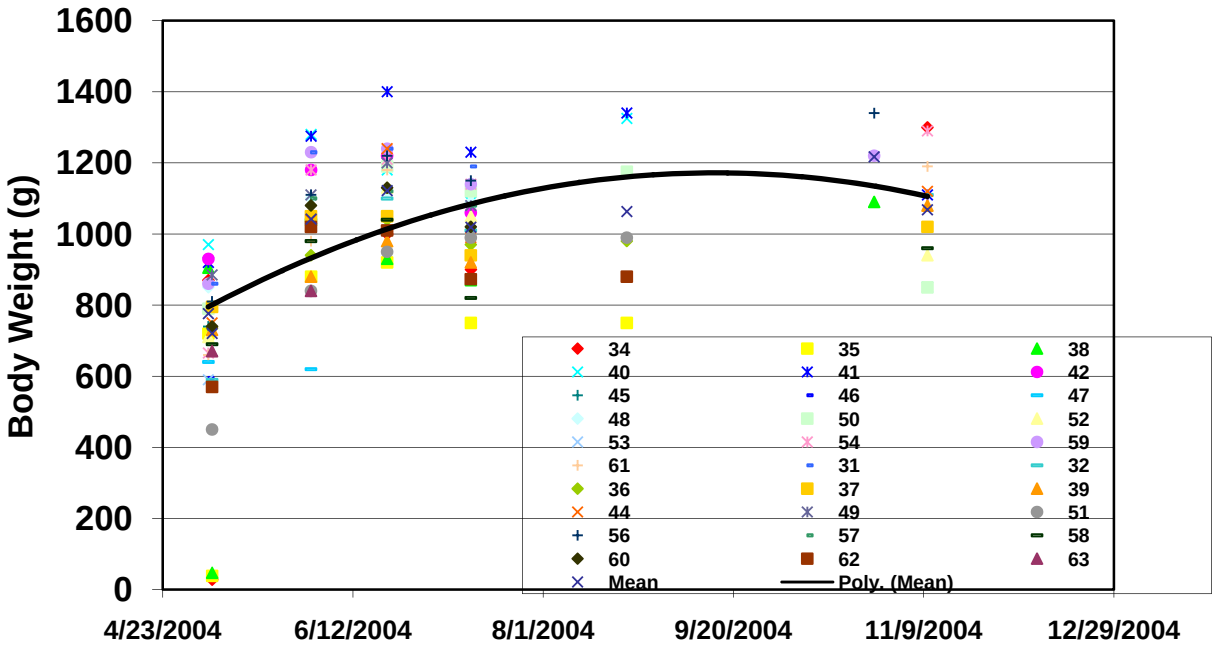


Figure 11. Mean body weight (g) for great egrets during Phase III (2004) study.