



Achieving environmentally relevant organochlorine pesticide concentrations in eggs through maternal exposure in *Alligator mississippiensis*

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Abstract

Alligator mississippiensis eggs from organochlorine pesticide (OCP) contaminated sites in Florida exhibit high rates of embryonic mortality compared to reference sites ($p < 0.05$). The objective of the present study was to use captive adult alligators to test the hypotheses that maternal exposure to OCPs results in increased OCP concentrations in eggs, and that increased exposure is associated with increased embryonic mortality. A total of 24 adult alligators (8 males and 16 females) were housed in eight pens. Eight females in four pens were dosed with a mixture of p,p'-DDE, toxaphene, dieldrin, and chlordane at a rate of 0.2 ± 0.01 mg/kg/day for 274 ± 8 days. Treated females produced eggs containing higher OCP concentrations ($12,814 \pm 813$ ng/g yolk) than controls (38 ± 4 ng/g yolk). Eggs of treated females exhibited decreased viability ($13 \pm 22\%$) as compared to controls ($45 \pm 20\%$). Results indicated that 0.6% of administered OCPs were maternally transferred to the eggs of American alligators, and that maternal exposure is associated with decreased egg/embryo viability in this species.

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In central Florida, American alligator (*Alligator mississippiensis*) eggs collected from organochlorine pesticide (OCP) contaminated sites (Lakes Apopka, Griffin, and Emerald Marsh) contain total concentrations of OCPs that range from 4000–30,000 ng/g yolk wet weight (not lipid normalized). This is several orders of magnitude greater than the control sites (Lakes Orange and Lochloosa) which exhibit concentrations of 231 ± 40 ng total OCPs/g yolk wet, weight (Heinz, Percival, & Jennings, 1991; Gross, unpublished data). In addition, alligator populations inhabiting OCP-contaminated sites have experienced increased embryonic mortality resulting in reduced clutch success (Masson, 1995; Rotstein et al., 2002). One possible explanation for these increased rates of embryonic mortality is embryonic exposure to OCPs, similar to effects reported in birds (Summer et al., 1996). The present study utilized a population of captive adult alligators to test the hypotheses that maternal exposure to OCPs would increase OCP burdens in egg yolks, leading to increased embryonic mortality and decreased hatch rates.

Alligators were obtained from JungleLand Zoo (Kissimmee, FL). Eight male and 16 female adult alligators, with mean snout-vent lengths of 119 ± 16 cm and mean weights of 53 ± 17 kg, were housed in eight pens (approximately 30 m \times 30 m) at a ratio of one male: two females. In addition, two treated females were housed in separate enclosures to monitor bioaccumulation of OCPs. Health status was monitored using monthly blood assessments of hematocrit, glucose, and total protein (Mader, 1996).

Selection of specific OCP analytes and dose calculations were based on OCP concentrations in alligator yolks collected from contaminated sites in Florida and avian maternal transfer rates (Donoghue, 2001; Huyghebaert, 2001). The dosing regime was designed to coincide with oocyte development and yolk formation (vitellogenesis), which for alligators begins in early Fall and continues through late Spring (Guillette et al., 1997; Lance, 1986). On 16 and 17 October, 2001, animals were randomized with treated individuals receiving one intramuscular (IM) and one intraperitoneal (IP) injection consisting of a mixture of p,p'-DDE (36.5 mg/kg), toxaphene (2.6 mg/kg), chlordane (2.5 mg/kg), and dieldrin (8.4 mg/kg) solubilized in reagent grade olive oil (cumulative injection volume of 40 ml). Control animals received the same volume of olive oil. OCP treatments resumed on 16 April, 2002, with treated animals being orally dosed with a cumulative mixture of p,p'-DDE (0.18 mg/kg), toxaphene (0.13 mg/kg), chlordane (0.014 mg/kg), and dieldrin (0.018 mg/kg). The cumulative doses for each chemical were equally divided and administered via diet at a rate of one administration per week over a 10-week period prior to oviposition. Control animals received the same feed ration minus the OCP mixture.

Four treated females were sacrificed to determine bioaccumulation rates of OCPs. Two of the females were housed in separate enclosures for monthly health status monitoring, and the other two were females which did not produce clutches. All four females were sacrificed within the same week as when the other treated females oviposited (24 June–10 July). Tissue samples (adipose, liver, and blood) were collected for analytical chemistry, along with one or two egg yolks from each of the females that oviposited. Tissues and yolks were screened for 30 OCP analytes by GC-MS. Lipid content (%) was determined gravimetrically for liver, and adipose,

while GC–MS techniques were used for blood (Furusawa, Ozaki, Nakamura, Morita, & Okazaki, 1999).

A total of six clutches were collected from three treated and three control females. Yolks collected from treated clutches had significantly higher ($p < 0.05$) mean total OCP concentrations ($12,840 \pm 813$ ng/g yolk wet weight) than control clutches (38 ± 4 ng/g yolk wet weight) (Table 1). Yolk burdens from the control group were less than those of the reference site (Orange/Lochloosa) (231 ± 30 ng/g). The treated group yielded yolk burdens ($12,840 \pm 813$ ng/g) that fell within the range of mean OCP concentrations (4000–30,000 ng/g) observed in contaminated sites (Apopka, Emeralda, and Griffin; Fig. 1(a)). Individual chemicals exhibited differing concentrations

Table 1
Organochlorine concentrations and blood chemistry values of captive adult female alligators

	Control	Treated
Yolk (ng/g ^a)		
p,p'-DDE	21 ± 3(3)	11,075 ± 6187(3)
Toxaphene	1 ± 1(3)	918 ± 1590(3)
Chlordane	8 ± 1(3)	112 ± 85(3)
Dieldrin	10 ± 1(3)	734 ± 343(3)
Lipid content (%)	19 ± 1(3)	21 ± 1(3)
Adipose tissue (ng/g ^a)		
p,p'-DDE	No data ^b	68,315 ± 35,275(4)
Toxaphene	No data ^b	8385 ± 1486(4)
Chlordane	No data ^b	708 ± 200(4)
Dieldrin	No data ^b	4372 ± 1237(4)
Lipid content (%)	No data ^b	82 ± 6(4)
Liver tissue (ng/g ^a)		
p,p'-DDE	No data ^b	8168 ± 3750(4)
Toxaphene	No data ^b	Not detected ^c
Chlordane	No data ^b	23 ± 13(4)
Dieldrin	No data ^b	143 ± 92(4)
Lipid content (%)	No data ^b	4 ± 2(4)
Whole blood (ng/g ^a)		
p,p'-DDE	No data ^b	179 ± 184(4)
Toxaphene	No data ^b	Not detected ^c
Chlordane	No data ^b	Not detected ^d
Dieldrin	No data ^b	15 ± 5(4)
Lipid content (%)	No data ^b	0.10 ± 0.02(4)
Hematocrit (%)	20–30 ^e	20 ± 4(4)
Glucose (mg/dl)	74 ^e	63 ± 17(4)
Total plasma protein (mg/dl)	5.1 ^e	6 ± 1(4)

Mean ± standard deviation (sample size).

^a ng chemical/g yolk wet weight (not lipid normalized).

^b No control females were sacrificed.

^c Limit of detection for toxaphene = 230 ng/g.

^d Limit of detection for chlordane = 0.2 ng/g.

^e Blood chemistry values reported by Mader (1996).

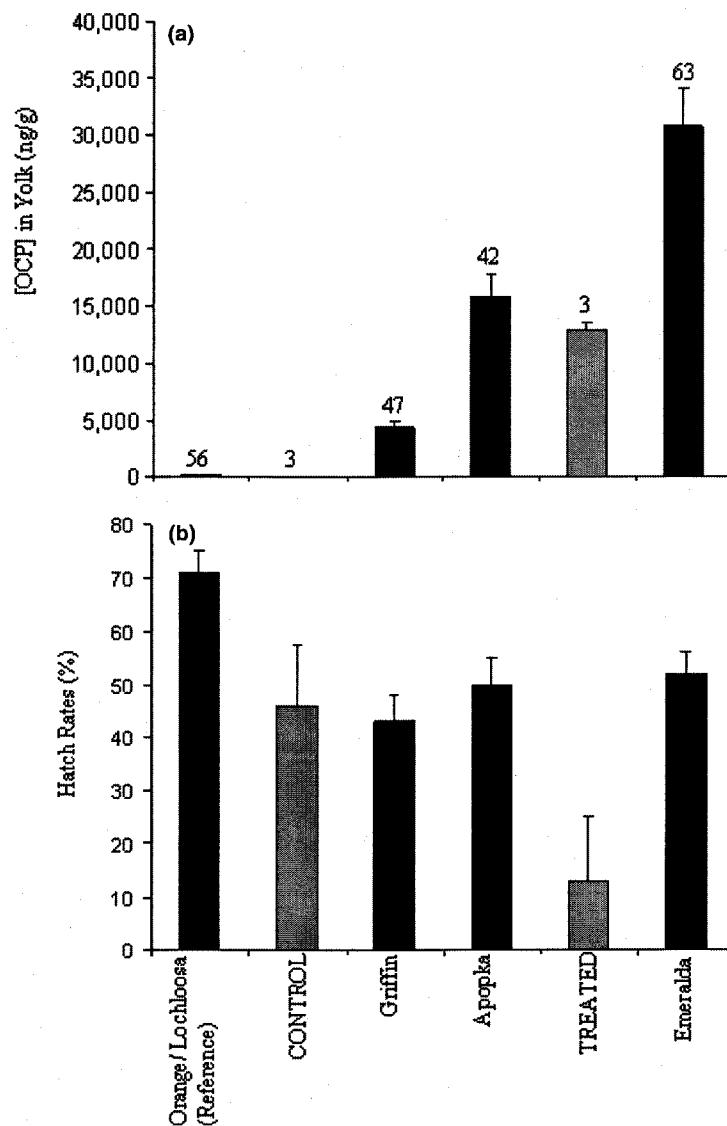


Fig. 1. (a) Total OCP concentrations (ng/g yolk wet weight) determined in alligator egg yolks. (b) Hatching % (number of hatchlings/total number of eggs in a clutch). Numbers above bars indicate number of clutches examined. Orange/Lochloosa = reference; Griffin, Apopka, and Emeralda = contaminated sites (Gross, unpublished data); CONTROL = captive-control population and TREATED = captive treated population (present study).

among tissues (Table 1). For OCP-treated animals, monthly health status assessments indicated that blood chemistry values were within normal ranges (Table 1). The percentage of the cumulative administered amount of OCPs (IP + IM + oral doses) that was transferred from the mother to the clutch was 0.6%. If the assumption is

made that all of the IP and IM doses were depurated by the time oral dosing began, then the percentage of the oral dose transferred to the clutch is 9.6%.

Hatch rates from clutches of captive control females ($46 \pm 20\%$) were significantly higher ($p < 0.05$) than those of treated females ($13 \pm 22\%$). These ranges fell within those observed in reference and OCP-contaminated Florida lakes (Fig. 1(b)).

The results of this preliminary study support our hypothesis that OCPs are maternally transferred to the developing egg, and that maternal exposure is associated with reduced clutch success and increased embryonic mortality. In addition, this is the first study to develop a method for exposing alligator embryos to endogenous concentrations of OCPs, in contrast to prior studies that have exogenously applied OCPs to eggs to elicit embryonic exposure (Matter, McMurry, Anthony, & Dickerson, 1998). Importantly, the dosing regime did not induce acute or chronic toxicity with respect to monthly blood chemistry assessments, food intake, weight gain, and behavior (e.g., females fiercely defended their nests). Maternal transfer rates of OCPs in the present study (0.6%) were less than those reported for PCBs in birds (22%) (Huyghebaert, 2001). Continuation of the present study will increase our understanding of the relationship between maternal exposure to OCPs and increased rates of embryonic mortality in the American alligator.

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