# CONTAMINANTS IN AMERICAN ALLIGATOR EGGS FROM LAKE APOPKA, LAKE GRIFFIN, AND LAKE OKEECHOBEE, FLORIDA

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**Abstract.** Residues of organochlorine pesticides, polychlorinated biphenyls (PCBs), and 16 elements were measured in American alligator (*Alligator mississippiensis*) eggs collected in 1984 from Lakes Apopka, Griffin, and Okeechobee in central and south Florida. Organochlorine pesticides were highest in eggs from Lake Apopka. None of the elements appeared to be present at harmful concentrations in eggs from any of the lakes. A larger sample of eggs was collected in 1985, but only from Lakes Griffin, a lake where eggs were relatively clean, and Apopka, where eggs were most contaminated. In 1985, hatching success of artificially incubated eggs was lower for Lake Apopka, and several organochlorine pesticides were higher than in eggs from Lake Griffin. However, within Lake Apopka, higher levels of pesticides in chemically analyzed eggs were not associated with reduced hatching success of the remaining eggs in the clutch. Therefore, it did not appear that any of the pesticides we measured were responsible for the reduced hatching success of Lake Apopka eggs.

## 1. Introduction

American alligators (*Alligator mississippiensis*) represent an important ecological and economic resource in Florida. Once endangered throughout their range, populations have recovered, and alligators are now raised commercially for meat and leather. The state of Florida regulates the taking of alligators, including the collection of alligator eggs from the wild for hatching in artificial incubators at commercial alligator farms.

For several years, while studying the effects of the taking of eggs and hatchlings on wild populations, we observed reduced hatching success of artificially incubated alligator eggs collected from Lake Apopka. Natural reproduction in Lake Apopka also declined in the early 1980's (Jennings *et al.*, 1988), and the incidence of deformed embryos is thought to have been greater in eggs from Lake Apopka than from other lakes (Allan Woodward, personal communication).

The potential for contaminant drainage into Lake Apopka comes largely from agricultural land, but some chemicals of industrial origin may have seeped into the lake as well. Our objectives were to (1) compare contaminant levels in alligator eggs from Lake Apopka with those from two other Florida lakes and (2) determine if concentrations of any of the contaminants we measured were correlated with the reduced hatching success of Apopka eggs.

## 2. Methods

In 1984, we collected two eggs from each of 7, 6, and 3 alligator nests on Lakes Okeechobee, Griffin, and Apopka, respectively, to provide a preliminary examination of contaminant levels. In this preliminary survey, we found eggs from Lakes Okeechobee and Griffin to be relatively clean and eggs from Lake Apopka the most contaminated. Therefore, in 1985 we chemically analyzed one egg from each of 21 nests on Lake Griffin and 23 nests on Lake Apopka. We used thickness index (Ratcliffe, 1967) to measure eggshell quality of the chemically analyzed eggs.

We artificially incubated the remaining eggs from each nest in 1985 to determine hatching success. Eggs were collected from nests, generally during the first trimester of incubation, and were carefully transported by boat and truck back to the laboratory for artificial incubation (Woodward *et al.*, 1989). Upon collection, a small dot was marked on the upward facing side of each egg so that this orientation could be maintained during artificial incubation. Eggs were placed in trays and packed with moist sphagnum or natural nest material and were incubated at 30-33 °C for the remainder of the 65-day incubation period.

We corrected contaminant levels for moisture loss from the egg (Stickel *et al.*, 1973). Organochlorine analyses of eggs collected in 1984 were conducted at the Patuxent Wildlife Research Center, following procedures described by Cromartie *et al.* (1975) and Kaiser *et al.* (1980). The lower reportable limit of residues on a wet-weight basis, before correction for moisture loss, was 0.1 ppm. Residues in about 10% of the eggs were confirmed by gas chromatography-mass spectrometry. For the 1985 samples, organochlorine analyses were carried out at Mississippi State Chemical Laboratory, Mississippi State, Mississippi, where results have been shown to be in close agreement with results from the Patuxent Wildlife Research Center.

In 1984, analyses for metals and other elements were conducted at Environmental Trace Substances Research Center, Columbia, Missouri. These analyses were not repeated in 1985 because no elements showed up at harmful levels in the 1984 samples. All elements, except mercury, arsenic, and selenium, were analyzed by inductively coupled plasma (ICP) techniques. Approximately 1 g of dried tissue was digested in nitric and perchloric acids and diluted to 100 mL. A 30-g sample of this solution was weighed into a 50-mL centrifuge tube. One mL of 2000 ppm Indium and 1 mL of 10% ammonium acetate buffer were added and the pH was adjusted to 6.5 with $NH_4OH$. One mL of 10% sodium diethyl dithiocarbamate was added. After mixing, the tubes were kept at 20 °C and were centrifuged at 15 000 rpm for 15 min. The liquid was decanted from the tubes and 0.3 mL of $HNO_3$ was added. The tubes were heated at 95 °C to dissolve the precipitate and diluted to 3 mL with deionized water. A Jarrell-Ash Model 1100 Mark III was used for the ICP analyses. The instrument was standardized with a series of seven standards containing 36 elements.

Mercury was analyzed by cold vapor atomic absorption. Samples were wet-digested in nitric acid. Stannous chloride was added to reduce ionic mercury to elemental mercury, which was measured photometrically in the vapor phase by atomic absorption. Arsenic and selenium were measured by hydride generation. Approximately 0.5 g of sample was digested with nitric and perchloric acids. Samples were refluxed overnight and then cooled. Two mL of HCl was added and the samples were heated. Samples were then removed from heat and diluted with deionized water. A Varian VGA-76 hydride generation accessory was mounted on either a Perkin-Elmer Model 603 or 3030 (B) atomic absorption spectrophotometer to measure selenium and arsenic.

The lower reportable limits on a wet-weight basis differed according to the element being analyzed and inter-element interference, but varied from about 0.004 to 0.8 ppm. For statistical purposes, when a residue was below the reportable limit, a value of one-half the reportable limit was assigned.

We used two-tailed *t*-tests to compare thickness index, pesticide residues, and hatching success of artificially incubated eggs from Lake Apopka to those from Lake Griffin. Because we have observed reproductive problems in eggs from Lake Apopka, we ran correlation analyses on data from that lake to search for relationships between hatching success of artificially incubated eggs and levels of each pesticide in the sample eggs. Residues were log transformed and data for percent hatch of eggs were subjected to angular transformations. A significance level of $\alpha = 0.05$ was used.

## 3. Results and Discussion

Although we collected eggs from only 16 nests in 1984, three findings were clear enough to help us plan our 1985 collections and analyses. First, organochlorine pesticides were uniformly highest in eggs from Lake Apopka (Table I). For this reason, we concentrated our efforts in 1985 on getting a large number of eggs from Lake Apopka and Lake Griffin, a relatively uncontaminated lake. In addition to the organochlorines listed in Table I, we also analyzed for, but did not find, *p*, *p*′-DDT, heptachlor epoxide, oxychlordane, or endrin.

Second, in 1984, there was close agreement between residues of organochlorines in pairs of eggs from the same nest. In Table II we list only the results for DDE, which was the most commonly found organochlorine, but there also was good agreement for the other organochlorines. Hall *et al.* (1979) reported close agreement in levels of organochlorines among eggs from the same clutch of the American crocodile (*Crocodylus acutus*); they attributed this agreement to the reptilian pattern of forming an entire clutch of eggs simultaneously. Birds, by contrast, form no more than one egg per day, which makes it possible for residues to vary from one egg to another depending on the contaminant level in the diet when each egg was being formed. Therefore, for alligators, and probably other reptiles as well, one egg is probably all that needs to be collected from each nest for contaminant analysis. For this reason, we collected only one egg from each nest in 1985. The remaining eggs should contain about the same contaminant burdens.

Third, in 1984, no metals or other elements were present at what appeared to be harmful levels; therefore, we did not repeat these analyses in 1985. Only aluminum, a relatively nontoxic metal, and iron and zinc, both essential trace elements, occurred at over 1 ppm (wet-weight) in eggs (Table III). It should be noted, however, that some metals do not

TABLE I

Pesticide and PCB residues in alligator eggs from Lake Okeechobee, Lake Griffin, and Lake Apopka in Florida, 1984

| Compound | Concentration (ppm, wet-weight) in egg | | |
|---|---|---|---|
| | Okeechobee ($n$=7)[a] | Griffin ($n$=6) | Apopka ($n$=3) |
| Toxaphene | ND[b][0][c] | 0.06[d] [2] (ND-0.07)[e] | 0.09 [2] (ND-0.13) |
| Dieldrin | ND[0] | 0.06 [2] (ND-0.10) | 0.24 [3] (0.10-0.52) |
| *p, p'*-DDE | 0.87 [7] (0.38-3.2) | 0.45 [6] (0.10-2.4) | 5.8 [3] (3.4-7.6) |
| *p, p'*-DDD | ND[0] | ND[0] | 0.82[3] (0.73-1.0) |
| *cis*-Chlordane | ND [0] | ND [0] | 0.09 [3] (0.07-0.13) |
| *trans*-Nonachlor | ND [0] | 0.05 [1] (ND-0.07) | 0.11 [3] (0.07-0.14) |
| *cis*-Nonachlor | ND [0] | ND [0] | 0.07 [1] (ND-0.11) |
| PCBs | ND [0] | 0.08 [1] (ND-0.67) | 0.17 [2] (ND-0.45) |

[a] $n$ = the number of nests sampled. Two eggs were taken from each nest; residues in these eggs were averaged to yield one value per nest.
[b] ND = not detected. The detection limit (on a wet-weight basis, before correction for moisture loss) was 0.10 ppm.
[c] Number of nests containing detectable residues.
[d] Geometric mean.
[e] Extremes.

readily accumulate in eggs; for these metals, samples of liver or kidney of adults may be a better indication of contamination.

In 1985, most organochlorine pesticides were significantly higher in alligator eggs from Lake Apopka than from Lake Griffin (Table IV). In addition to the organochlorines listed in Table IV, we analyzed for, but did not find, hexachlorobenzene, hexachlorocyclohexane, heptachlor epoxide, PCBs, *o, p'*-DDD, *o, p'*-DDT, endrin, *cis*-nonachlor, mirex and dicofol (plus its metabolites).

There are few published reports of contaminants in alligator eggs with which we can compare our results from Lake Apopka. Ogden *et al.* (1974) collected four American alligator eggs from Everglades National Park in 1972; on a wet-weight basis, these eggs contained means of 2.42 ppm DDE, 0.30 ppm PCBs, and 0.69 ppm mercury, plus much lower levels of other organochlorines and metals. Due to a scarcity of information on the chronic effects of environmental contaminants on reptiles (which is a problem that still exists today), they were unable to give much interpretation to the residues they found.

In 1985, Delany *et al.* (1988) measured contaminants in muscle of alligators from eight different lakes in Florida, including Lake Apopka. Levels of metals were low in muscle

TABLE II

DDE residues in two alligator eggs taken from each of several nests on Lake Okeechobee, Lake Griffin, and Lake Apopka in Florida, 1984

| | | Concentration (ppm, wet weight) in egg | | |
|---|---|---|---|---|
| Nest number | Egg number[a] | Okeechobee | Griffin | Apopka |
| 1 | 1 | 0.48 | 0.10 | 7.1 |
| | 2 | 0.49 | 0.10 | 7.6 |
| 2 | 1 | 0.33 | 0.50 | 8.1 |
| | 2 | 0.44 | 0.57 | 7.2 |
| 3 | 1 | 0.46 | 1.5 | 3.7 |
| | 2 | 0.32 | 1.2 | 3.2 |
| 4 | 1 | 2.9 | 2.4 | |
| | 2 | 3.6 | 2.4 | |
| 5 | 1 | 2.3 | 0.14 | |
| | 2 | 2.1 | 0.13 | |
| 6 | 1 | 1.2 | 0.37 | |
| | 2 | 1.2 | 0.31 | |
| 7 | 1 | 0.67 | | |
| | 2 | 0.58 | | |

[a] Egg number does not refer to the sequence in which these eggs were laid; these numbers were arbitrarily assigned.

samples from Lake Apopka, just as they were in the eggs we collected. Except for PCBs, which had a mean of 2.1 ppm on a wet-weight basis, organochlorines were reported at less than 0.2 ppm. We cannot explain the absence of PCBs in our eggs when they were reported in the muscle samples. No DDE was reported in muscle. Muscle, owing to its lower lipid content, would be expected to contain less DDE than eggs, but its complete absence is surprising, especially since DDT, the parent compound, was found.

One surprising finding in our own data was the difference in toxaphene residues in 1984 versus 1985. Nearly all uses of toxaphene were cancelled in 1982 by the U.S. Environmental Protection Agency (Environmental Protection Agency, 1982). However, there were provisions for certain minor uses and for using up existing stock. It is, therefore, possible that toxaphene could have been used on surrounding crop land in 1985, but not in 1984. Another possible explanation is that toxaphene is a complex mixture of chlorinated camphenes and quantification is difficult and varies among laboratories.

Some factor appears to be depressing hatching success of alligator eggs at Lake Apopka because, in 1985, success (mean $\pm$ SE) in 23 nests there was only 19.3 $\pm$ 5.42%; this was significantly lower than the 46.4 $\pm$ 7.97% for 20 nests in Lake Griffin ($P$=0.01). None of the organochlorine pesticides appear to be this factor, however, because none produced a significant negative correlation between pesticide level in the sample egg and hatching success of the remaining eggs in the clutch. Had any pesticides been responsible for poor hatching at Lake Apopka, we would have expected that hatching success would have gotten worse as pesticide residues in sample eggs increased.

TABLE III

Concentrations of metals and other elements in alligator eggs from Lake Okeechobee, Lake Griffin, and Lake Apopka in Florida, 1984

| | Concentration (ppm, wet-weight) in egg | | |
|---|---|---|---|
| Element | Okeechobee ($n$=7)[a] | Griffin ($n$=6) | Apopka ($n$=3) |
| Aluminium | 1.5[b] [7][c] (0.90-2.8)[d] | 2.0 [6] (1.7-3.0) | 1.3 [3] (0.86-2.0) |
| Arsenic | ND[e] [0] | ND [0] | ND [0] |
| Beryllium | ND [0] | ND [0] | ND [0] |
| Cadmium | ND [0] | ND [0] | ND [0] |
| Chromium | 0.09 [6] (ND-1.2) | 0.08 [6] (0.04-0.17) | 0.09 [2] (ND-0.38) |
| Copper | 0.32 [7] (0.12-0.73) | 0.78 [6] (0.70-0.86) | 0.52 [3] (0.36-0.63) |
| Iron | 13 [7] (9.2-22) | 13 [6] (11-14) | 11 [3] (9.0-13) |
| Lead | 0.14 [1] (ND-0.90) | 0.22 [3] (ND-0.62) | ND [0] |
| Manganese | 0.14 [7] (0.10-0.26) | 0.15 [6] (0.11-0.20) | 0.14 [3] (0.10-0.18) |
| Mercury | ND [0] | ND [0] | ND [0] |
| Molybdenum | ND [0] | ND [0] | ND [0] |
| Nickel | 0.07 [7] (0.02-0.64) | 0.05 [6] (0.03-0.14) | 0.09 [3] (0.03-0.20) |
| Selenium | 0.31 [7] (0.24-0.38) | 0.37 [6] (0.29-0.53) | 0.30 [3] (0.25-0.44) |
| Thallium | ND [0] | ND [0] | ND [0] |
| Vanadium | ND [0] | ND [0] | ND [0] |
| Zinc | 6.7 [7] (4.9-9.2) | 7.6 [6] (6.6-8.2) | 5.6 [3] (5.4-5.8) |

[a] $n$ = the number of nests sampled. Two eggs were taken from each nest; residues in these eggs were averaged to yield one value per nest.
[b] Geometric mean.
[c] Number of nests containing detectable residues.
[d] Extremes.
[e] ND = not detected (on a wet-weight basis, before correction for moisture loss) at the following concentrations: arsenic (0.05 ppm), beryllium (0.004 ppm), cadmium (0.01 ppm), chromium (0.04 ppm), lead (0.20 ppm), mercury (0.03 ppm), molybdenum (0.03 ppm), thallium (0.80 ppm), and vanadium (0.01 ppm).

DDE was the most prevalent organochlorine in eggs from Lake Apopka, and is a chemical known to cause eggshell thinning and reproductive failure in birds. Alligators, like birds, have a hard, calcified eggshell, so we felt it was important to look for DDE-induced shell thinning. There was, however, no significant difference between the mean thickness index of Lake Apopka eggs (2.91) and those from Lake Griffin (2.90). In addition, if DDE were causing eggshell thinning in Lake Apopka, thickness index in sample eggs should have decreased as DDE residues increased, but the correlation between the two was not only not statistically significant, but was positive ($r$=+0.12).

TABLE IV

Comparison of pesticide concentrations in alligator eggs from Lake Griffin and Lake Apopka in Florida, 1985

| Compound | Concentration (ppm, wet-weight) in egg | |
|---|---|---|
| | Lake Griffin, $n$=21 | Lake Apopka, $n$=23 |
| Toxaphene | 1.1[a] [21][b] (0.33-4.0)[c] | 2.4 [23]* (0.05-13) |
| Dieldrin | 0.05 [21] (0.01-0.14) | 0.11 [23]* (0.02-1.0) |
| *p, p′*-DDE | 0.58 [21] (0.19-3.8) | 3.5 [23]* (0.89-29) |
| *p, p′*-DDD | 0.007 [2] (ND[d]-0.35) | 0.37 [22]* (ND-1.8) |
| *p, p′*-DDT | ND [0] | 0.02 [6]* (ND-1.3) |
| *o, p′*-DDE | 0.05 [20] (ND-0.13) | 0.007 [4]* (ND-0.06) |
| *trans*-Chlordane | 0.02 [20] (ND-0.05) | 0.006 [2]* (ND-0.05) |
| *cis*-Chlordane | 0.03 [20] (ND-0.09) | 0.06 [22]* (ND-0.25) |
| *trans*-Nonachlor | 0.09 [21] (0.04-0.20) | 0.15 [23]* (0.01-0.68) |
| Oxychlordane | 0.03 [21] (0.01-0.07) | 0.03 [22] (ND-0.21) |

[a] Geometric mean.
[b] Number of eggs containing detectable residues.
[c] Extremes.
[d] ND = not detected. The detection limits (on a wet-weight basis, before correction for moisture loss) were 0.05 ppm for toxaphene and 0.01 ppm for all others.
* The geometric mean for Lake Apopka was significantly different from the mean for Lake Griffin at $\alpha = 0.05$ using two-tailed *t*-tests.

Hall *et al.* (1979) also found no correlation between DDE residues and shell thickness in American crocodiles at Everglades National Park in Florida, but the crocodile eggs contained a mean of only 1.19 ppm DDE and ranged from 0.37 to only 2.9 ppm. For Lake Apopka alligator eggs, the mean DDE level in 1985 was 3.5 ppm, and ranged from 0.89 to 29 ppm. This range should have been great enough to have shown a significant correlation between DDE and shell thickness had alligators been as sensitive to this effect as are the most vulnerable bird species.

For brown pelicans (*Pelecanus occidentalis*), which are among the most sensitive of birds to DDE-induced shell thinning, 15% shell thinning was associated with between 4 and 5 ppm DDE in eggs on a wet-weight basis, and reproductive impairment with concentrations above 2.5 ppm (Blus *et al.*, 1974). We cannot conclude that alligators are not at all sensitive to DDE-induced shell thinning, because even with black ducks (*Anas rubripes*), which are fairly sensitive to DDE, eggshell thinning in one controlled experiment was associated with a mean of 17.9 ppm DDE in eggs (Longcore and Stendell, 1982), indicating considerable variability, even among sensitive birds.

In 1985, the Florida Department of Agriculture and Consumer Services analyzed fish samples from Lake Apopka for organophosphate pesticides, but found none (W. George Fong, personal communication); therefore, we can probably also rule out this group of pesticides as having been a cause of poor hatching.

We cannot rule out the possibility that some unmeasured pesticide or industrial chemical has been causing reduced hatching of Lake Apopka alligator eggs. Although it is impractical to further analyze Lake Apopka alligator eggs for all known man-made contaminants, should evidence appear that some other toxic chemical has entered the lake at high levels, additional contaminant work would be warranted. In addition, it is possible that some contaminant could be altering the physiology of adult alligators such that their eggs are adversely affected. Therefore, we recommend that if future contaminant research be done on Lake Apopka alligators, it concentrate on searching for additional contaminants as well as contaminant effects on adults. Other possible causes for the poor hatching success of Lake Apopka alligator eggs, such as disease, nutrition, and inbreeding, also need to be studied.

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