Endocrine Disruptors and Host Resistance in Lake Apopka Alligators

Project Scope

Declines in populations of wildlife species, such as the American alligator, in Lake Apopka, FL, have been associated with reproductive effects of endocrine-disrupting environmental contaminants (EDCs), primarily p,p'-DDE but also including chlordane, dieldrin, endosulfan, methoxychlor, and toxaphene. Recently, concern has arisen that EDCs can have other adverse effects, including altered immunity and decreased resistance to disease. Organisms may be especially susceptible to such effects during stages of embryonic and fetal development.

In the early 1980s, the population of juvenile alligators in Lake Apopka declined by 90 percent in association with decreased egg viability, increased neonatal mortality, and increased organochlorine pesticide contamination. Hypoplastic lymphoid organs and bone marrow and weakened antibody responses in juvenile Lake Apopka alligators were observed, suggesting that weakened host defenses could be a contributing factor leading to their decline.

The main objective of this research was to study the effects of EDCs on neonatal alligators by evaluating measures of specific and nonspecific immunity as well as lymphoid organ and bone marrow development. To address this objective, researchers studied the following groups:

- *Treated*: alligators hatched from eggs obtained from minimally contaminated reference sites (Agrifos Collective, Tampa, FL, and Rockefeller National Wildlife Refuge, Grand Chenier, LA) that were then treated with several of the same EDCs as found in tissues and eggs of Lake Apopka alligators.
- *Naturally exposed*: alligators hatched from eggs obtained from various Florida lakes naturally exposed to EDCs (including North Lake Apopka, South Lake Apopka, Lake Griffin, Demonstration Marsh, and Emeralda Marsh Conservation Area). These lakes represent a range of EDC contamination.
- *Controls*: alligators hatched from eggs obtained from minimally contaminated reference sites (Orange Lake and Lake Woodruff National Wildlife Refuge) that were untreated.

Grant Title and Principal Investigator

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Trenton R. Schoeb, University of Florida

EPA STAR Grant #R826127

Key Findings

- There appear to be no statistically significant effects of pesticide treatments of eggs or level of natural contamination with EDCs among different lakes on the following measures of immune response in American alligators: peripheral blood antibacterial activity against *Staphylococcus aureus*; histology of the spleen, thymus, or bone marrow; spleen weight/body weight ratios; or in vivo lymphocyte response to phytohemagglutinin.
- A significant finding of unknown cause was the presence of focal areas of necrosis in the brains of 6 out of 31 necropsied young animals. Affected animals were either observed with neurologic signs, including ataxia and loss of righting ability, or were found moribund or dead. The affected animals were from Lakes Apopka and Griffin and came from clutches with low clutch viability. Additional studies are necessary to determine whether there is an association with EDC exposure and these brain lesions in alligators.
- There appears to be no evidence of a relationship between clutch viability and exposure to any of the pesticides in organochlorine analyses at the doses examined. This is a significant observation indicating that factors other than pesticide exposure are likely contributing to embryonic mortality and clutch viability that is observed in the free-living alligator populations.

Project Period: January 1998 to January 2001

Reference sites were identified as having historically lower levels of contamination in sediments and alligator eggs than other Florida lakes according to findings by the United States Geological Survey (USGS) and Florida Fish and Wildlife Conservation Commission (FFWCC) investigators.

Study measures included: (1) ability to generate T cell-dependent humoral immune responses; (2) various hematologic parameters; (3) effects on peripheral blood antibacterial activity; (4) changes in lymphoid organ or marrow cell morphology; and (5) susceptibility to the *Mycoplasma alligatoris* pathogen.

This study also examined whether embryos naturally exposed to EDCs had increased numbers of bacterial and fungal isolates in yolk samples. These studies were conducted at the following locations in Florida: Lake Griffin, Lake Apopka, Emeralda Marsh Conservation Area, Lake Jessup, Lake Okeechobee, Lake Hancock, Lake Monroe, Orange Lake (control), and Lake Woodruff (control). Increased numbers of naturally-occurring bacteria or fungi in EDC-exposed eggs may indicate an impaired immune response due to EDCs (or other factors). In addition, researchers evaluated the potential causes of morbidity and mortality in pesticide-exposed perinatal alligators.

Most previous research on endocrine disruptors has focused on reproductive and endocrine effects. The present work addressed concerns that EDCs could have adverse effects on the immune system, especially during development, and that such effects could adversely affect the overall health of wildlife species (not limited to Lake Apopka alligators).

Relevance to ORD's Multi-Year Research Plan

This project contributes directly to two important long-term goals of the ORD's MYP: (1) to provide a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors, and (2) to determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment.

Much of the research associated with the effects of EDCs on wildlife has focused on effects related to reproduction. The purpose of this study was to examine the potential effects of EDC exposure on the immune system of American alligators in Florida. It was hypothesized that impaired immune system response, another purported effect of EDCs, may be responsible for the observed low hatching success of eggs and high mortality rate of young alligators in the Ocklawaha River basin area of Florida, which is contaminated with a variety of organochlorine pesticides.

This study found no statistically significant effects of pesticide treatments or level of different lake contamination with EDCs on various measures of immunity in embryonic, perinatal, or neonatal alligators. However, brain lesions were found in over 20 percent of the young alligators hatched from eggs from two of the most contaminated lakes. Additional study will be required to determine the cause of these lesions.

Project Results and Implications

Egg Collection. In 1998, American alligator eggs were obtained from minimally contaminated reference sites (Agrifos Collective and Rockefeller National Wildlife Refuge). The eggs were distributed in a randomized block fashion into nine treatment groups, including: dimethyl sulfoxide (DMSO) control, chlordane, p,p'-DDE, dieldrin, endosulfan, methoxychlor, toxaphene, and both a low and a high dose combination of the six chemicals.

Eggs also were collected from the Ocklawaha River basin in Florida. Eggs obtained from Lake Apopka, Lake Griffin, Demonstration Marsh, and Emeralda Marsh Conservation Area represented naturally exposed eggs exhibiting a range of EDC contamination. Selection of these contaminated lakes also was based on USGS and FFWCC data, except for Lake Griffin, which was included because of more recent evidence of increased adult alligator mortality and decreased egg viability. Eggs collected from Orange Lake and Lake Woodruff represented the untreated controls for the River basin.

In 1999, American alligator eggs were obtained from the Rockefeller National Wildlife Refuge. The eggs were distributed in a randomized block fashion into five treatment groups. Eggs within eight days of oviposition were injected with the DMSO vehicle control, 1, 5, or 25 ppm p,p'-DDE or a combined solution of 15 ppm p,p'-DDE, 100 ppb chlordane, 100 ppb dieldrin, 100 ppb methoxychlor, and 3 ppm toxaphene based on average egg weight. Eggs also were obtained from Orange Lake, North Lake Apopka, South Lake Apopka, Lake Griffin, and Emeralda Marsh Conservation Area. The exposure doses were calculated to produce concentrations in eggs that would be generally in the range of those found in highly contaminated sites; most concentrations were representative of the more heavily contaminated Florida lakes rather than the "typical" concentrations at any particular site.

Analyses. Liver samples from hatchlings in each experimental group were analyzed. Analyses verified the absorption and presence in tissues of DDE, chlordane, dieldrin, and toxaphene in the treated and naturally exposed groups. Endosulfan and methoxychlor were not present in detectable amounts, likely because they are subject to metabolism by the liver. DDE and dieldrin were detected in reference site groups, in the 5 - 50 ppb range.

T cell-dependent antibody responses were assessed by immunization with keyhole limpet hemocyanin (KLH) esterified with dinitrophenol (DNP) (DNP-KLH). DNP hapten-specific responses were measured by enzyme-linked immunosorbent assay (ELISA). No significant differences in T cell-dependent antibody response were found among experimental groups or lake study sites. There were no statistically significant differences among the experimental groups or lake study site groups in white or red blood cell counts, packed cell volumes (hematocrits), plasma protein, or hemoglobin, except for a higher mean heterophil count in the Emeralda Marsh group and a lower mean heterophil count in the Lake Griffin group, neither of which was considered clinically significant. In addition, no statistically significant effects of experimental groups or lake groups were found for the following: peripheral blood antibacterial activity against *Staphylococcus aureus*; histology of the spleen, thymus, or bone marrow; spleen weight/body weight ratios; or *in vivo* lymphocyte response to phytohemagglutinin.

Three-month old alligators from control and DDE treatment groups were inoculated intravenously with the alligator pathogen *Mycoplasma alligatoris*. Blood, brain, and pericardium were cultured, and joints, brain, and any tissues with gross lesions were prepared for histologic examination. There were no statistically significant differences in quantitative culture results among treatment or lake site groups. Joint and brain lesion scores determined by histologic examination were not significantly different among the treatment or lake site groups. However, there was a non-significant trend toward decreased joint lesion severity in the higher dose DDE groups (see Figure 1). This possibly decreased lesion severity could indicate a reduced host immune response in the higher DDE dose groups. Additional studies with more animals are necessary to evaluate that hypothesis.

Egg microbiology was studied to determine if alligator embryos exposed to EDCs had increased numbers of bacterial or fungal species in yolk compared to alligators from a relatively uncontaminated lake. Subsets of eggs from clutches at Lake Griffin, Lake Apopka, Emeralda Marsh Conservation Area, Lake Jessup, Lake Okeechobee, Lake Hancock, Lake Monroe Orange Lake, and Lake Woodruff were collected for a total of 151 eggs. The remaining eggs from each clutch were incubated at approximately 32^{o}C until hatching, and clutch viabilities were determined. Both gram-positive and gram-negative aerobic bacteria (68 species) were isolated from yolk. Fungal isolates were rare. Species for which occurrence was statistically lower in viable eggs included *Pseudomonas aeruginosa*, *Seratia marcescens*, *Proteus vulgaris*, *Brevundimonas diminuta*, and *Chromobacterium violaceum*. There was no evidence of a relationship between the total number of aerobic bacteria species in yolk and a particular lake site or clutch.

There was no evidence of a relationship between clutch viability and exposure to any of the pesticides in organochlorine analyses at the doses examined. This is a significant observation indicating that factors other than pesticide exposure are likely contributing to embryonic mortality and clutch viability in the free-living alligator populations.

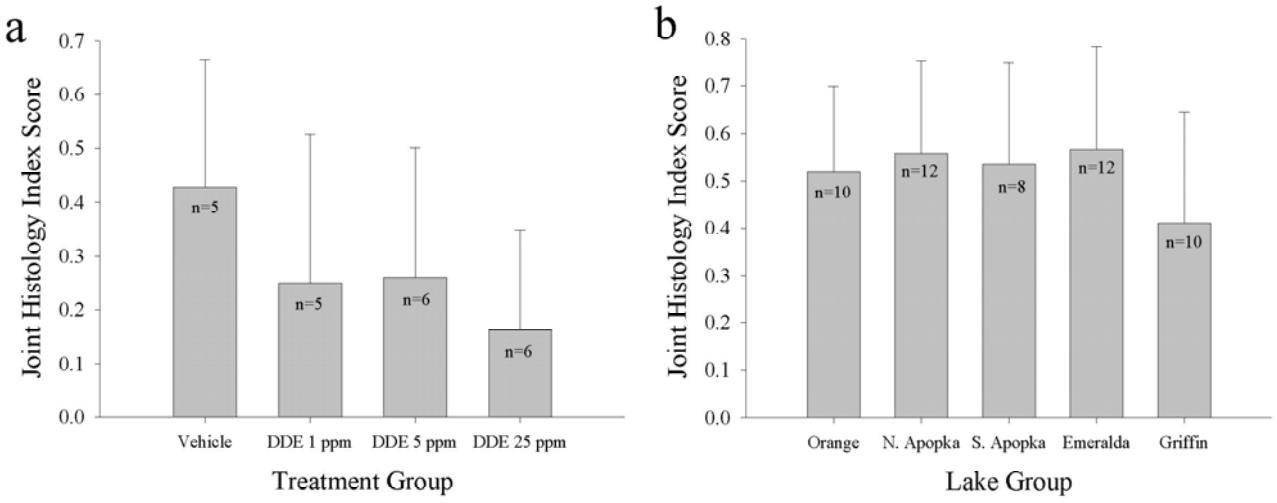

Figure 1. Joint lesion severity indices for combined tarsal and carpal joints at 28 dpi (mean ± SD). Higher indices indicate more severe lesions. Three-month-old alligators were inoculated intravenously with 10^6 colony-forming units *Mycoplasma alligatoris* ATCC 700619 and sacrificed 28 days later. Lesion indices are sums of scores of 0-4 each for cartilage erosion, subchondral bone changes, exudate, and synovial lesions divided by the maximum possible total score. Animals that died or were euthanized prior to 28 dpi were excluded. (a) DDE study (no significant differences, p = 0.3414); (b) Lake study (no significant differences, p = 0.4588). (From Richey LJ, Effects of endocrine-disrupting contaminants on the immune system of hatchling American alligators, PhD dissertation, University of Florida, 2001).

Alligators naturally exposed to EDCs in Florida lakes were studied by gross and/or microscopic examination to determine whether morbidity and mortality were associated with infections that could provide evidence of compromised immune systems. Of the more than 4,300 eggs incubated, 48 perinatal hatchlings were necropsied. These 48 either died shortly before hatching or shortly after hatching or were sacrificed after hatching due to obvious illness. Indicators of *in ovo* disease included yolk sacculitis with associated bacteria, inflammation of the albumen remnant with associated fungal hyphae and bacteria, and failure of the heart to migrate into the body cavity with associated severe chronic-active fibrinopurulent pericarditis. Livers of the necropsied animals were not analyzed for specific contaminant levels.

The lesions in neonatal alligators included suppurative or pyogranulomatous and ulcerative inflammation of the gastrointestinal tract. Many of these lesions were severe and could have contributed to mortality. Other lesions included multifocal bacterial hepatitis, suppurative pyelonephritis, suppurative adrenalitis, suppurative interstitial pneumonia, and pyogranulomatous bacterial choroiditis and ventriculitis. These lesions were likely related to bacterial infection. From the embryos and neonates sampled for aerobic bacteria, 39 bacterial species, often-opportunistic pathogens, were isolated with no consistent isolates relating to mortality. A significant finding of unknown cause was the presence of focal areas of necrosis in the brains of 6 out of 31 animals. Affected animals were either observed with neurologic signs, including ataxia and loss of righting ability, or were found moribund or dead. The affected animals were from Lakes Apopka and Griffin and came from clutches with low clutch viability. Because more than 20 percent of the animals examined had this lesion, and because animals from these lakes were observed in past years with similar clinical signs, this lesion may play a significant role in the mortality of neonates, and possibly embryos, from these lakes. Possible causes of the lesions include: toxicants other than organochlorine pesticides, such as lead, selenium, methyl mercury, trimethyl and triethyl tin, kainic acid, domoic acid, vascular disease (thromboembolism, infarcts); and nutritional disease (thiamine deficiency). Similar lesions were observed in the brains of adult Lake Griffin alligators that were found sick or moribund. Although organochlorine contaminants have not been associated with such lesions in birds and mammals, additional studies are necessary to determine whether there is an association with EDC exposure and these brain lesions in alligators.

Investigators

T.R. Schoeb, University of Florida
M.B. Brown, University of Florida
T.S. Gross, U.S. Geological Survey
P.A. Klein, University of Florida

For More Information

http://www.flmnh.ufl.edu/natsci/herpetology/lakes/lakes.htm
http://www.tmc.tulane.edu/ecme/eehome/,http://www.epa.gov/endocrine/links.html

NCER Project Abstract and Reports:

http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/174/report/F