

A proposal entitled:

ASSESSMENT OF OSPREY (*PANDION HALIAETUS*) AND GREAT WHITE HERON
(*ARDEA OCCIDENTALIS*) EXPOSURE TO ORGANOCHLORINES IN THE
EVERGLADES NATIONAL PARK UTILIZING A NON-LETHAL AND NON-
INVASIVE METHOD OF ASSESSMENT

by

Tim Bargar

The Institute of Environmental and Human Health
Department of Biological Sciences
Texas Tech University
Lubbock, Texas 79409

December 7, 1998

BACKGROUND

Assessment of environmental pollutant levels in sentinel species requires residue analysis of environmental samples. Exposure levels are usually determined by sampling various environmental media and then predicting levels based on the likelihood and probability of exposure to the sampled media. Pollutant levels in aquatic organisms can be confidently predicted from sampling water since one of the major routes of pollutant uptake from water is by direct absorption. Pollutant levels in terrestrial organisms would be more difficult to predict based on water sampling since they do not frequently directly contact water. Therefore, prey and tissue samples are taken to ascertain pollutant levels in terrestrial organisms. Although the likelihood of exposure to prey items is great, pollutant metabolism and excretion by both prey items and the sentinel species make it difficult to predict burdens in the sentinel species based on pollutant levels in prey items. Directly obtaining tissue samples from the sentinel species alleviates metabolism and excretion influence upon pollutant level predictions. It gives a direct measure of pollutant levels in the organism. However, handling stresses from invasive sampling methods and organism capture difficulties are potential drawbacks to tissue sampling. A non-invasive tissue sampling method would be ideal for pollutant level determination in sentinel species.

The chorioallantoic membrane (CAM) is a tissue that may be non-invasively sampled for pollutant level determination in oviparous organisms. The CAM is necessary during embryonic development because it serves as the respiratory organ. However, it is discarded at hatch because the lungs assume the respiratory role shortly before hatch. The CAM frequently remains in the discarded shell and may be easily sampled by collecting the shells. The question then becomes are pollutant levels determined by CAM residue analysis indicative of pollutant levels in the newly hatched organism and adult?

CAMs have been investigated as tools for use in exposure assessments. CAMs from discarded great blue heron (*Ardea herodias*) eggshells were analyzed for polychlorinated biphenyls (PCBs) and dioxins to ascertain chemical levels in adult herons (Cobb et al. 1995, 1994). They found both PCBs and dioxins leading the authors to suggest organochlorine levels in adult birds may be screened by sampling CAMs. Additionally, since numerous CAMs were obtained by collecting discarded shells, they suggested it would be an assessment method that would decrease uncertainty in predicting organochlorine levels in birds. In other studies, CAMs and embryos from inviable american alligator (*Alligator mississippiensis*) and loggerhead sea turtle (*Carretta carretta*) eggs were analyzed for PCBs (Cobb and Wood, 1997a, 1997b). PCB levels in CAMs were significantly correlated with PCB levels in dead embryos. Although site contamination levels were not determined in these studies, residue levels in CAMs and whole eggs were indicative of greater soil and sediment residue levels as determined in previous studies. The authors concluded that total PCB levels in whole eggs may be accurately predicted from knowing total PCB levels in CAMs and that chemical bioaccumulation may also be determined. Neither of these studies investigated residue

levels in living organisms in relation to residue levels in CAMs. Physicochemical properties of compounds can alter the excretion of those chemicals from the adult into the egg and the absorption of those chemicals from the yolk by the embryo leading to chemical profiles in those three compartments which are different. This information would be helpful in understanding the relationship of chemical presence in the CAM to chemical presence in the egg and adult.

CURRENT STUDIES

A laboratory study with White leghorn chickens (*Gallus domesticus*) investigated PCB and endosulfan presence in egg yolks as a function of the compound's physical properties. It was found that physical the properties of the chemicals influence transfer of the chemical into the egg. An average of 0.64% to 0.42% of the total PCB congener mass injected into the hen was extracted from the egg yolk depending on the chlorine substitution pattern. An average of 0.12% to 0.02% of injected endosulfan was extracted from the yolk depending on the isomer. PCBs are also more persistent chlorinated organics relative to endosulfan. This could be the reason for the differences between the percentage of PCB found in yolks relative to endosulfan. Therefore, physicochemical parameters affect maternal transfer of contaminants into the egg.

In a second study, adult hens were dosed with increasing concentrations of the same compounds to determine if a dose dependent relationship existed between contaminant mass in the adult and contaminant mass in the CAM. The total PCB mass extracted from CAMs was significantly correlated with the mass that was injected into the adults ($r^2=0.676$). Therefore, CAM analysis could be used to determine adult exposure to persistent contaminants. The more transient organochlorine (OC) pesticide endosulfan was not detected in CAM extracts. As a result, sampling CAMs may be used to assess the risk to birds due to their exposure to persistent contaminants at variably contaminated sites.

A concurrent study investigated contaminant mass balance in developing chick embryos. Just as chemical physical properties affected their transfer from the adult into the egg, the same properties affects their absorption from the yolk into the embryo. Between 90% to 100% of the total chemical mass in the egg remains within the yolk and albumin through two-thirds of embryonic development. By 90% of embryonic development, significant chemical transfer into the embryo had taken place. Either 9% (a-isomer) or 30% (b-isomer) of the total endosulfan mass in the egg had moved into the embryo. Either 2% (a-isomer) or 8.5% (b-isomer) of the total endosulfan mass had been transported into the CAM. Depending on chlorination, from 32% to 42% of the total PCB mass in the egg had been transported into the embryo while 0.2% to 3.5% of the total mass had been transported into the CAM. Two important findings from this study should be noted. First, the majority of contaminants within the egg remain within the yolk and albumin through at least 90% of embryonic development. Anywhere from 55% to 90% of chemicals within the egg remained external to the embryo meaning the embryo is not exposed to the majority of contaminants within the egg through the majority of it's development. Second, the chemical's physical properties influence embryonic absorption

of contaminants indicating whole egg residue analysis may not accurately represent embryonic exposure to contaminants.

These studies have shown adult chemical body burdens may be estimated from determination of chemical burdens in eggs and CAMs. Based on the chemicals utilized in these studies, the adult body burden will be around 200 fold greater than the mass in the yolk and 1,000 to 10,000 fold greater than the mass in the CAM. However, it was shown that the chemical's physical properties affect chemical transfer meaning that the difference between CAM or egg chemical levels and adult chemical levels may differ among chemicals. Therefore, the factors that affect chemical transfer from the adult into the egg and from the yolk into the embryo must be understood in order to determine relative levels for different chemicals. Also, determination of whole egg chemical levels may overestimate embryonic exposure be inaccurate. Sampling CAMs would be a good alternative to whole egg analysis for assessment of embryonic exposure because chemicals must be absorbed and pass through the embryo before entering the CAM.

OBJECTIVES

Organochlorine (OC) residue levels will be determined in inviable eggs, blood, and CAMs of great white herons (*Ardea occidentalis*) and osprey (*Pandion haliaetus*). Both species feed largely on aquatic organisms in the proposed study region making them suitable sentinel species to determine if lipophilic contaminants present in the aquatic system biomagnify in piscivorous birds. Inviably eggs will be analyzed because they are routinely used in avian exposure assessments and can serve as a basis to which blood and CAM contaminant levels may be compared. The other two sample types are alternatives to egg analyses. They represent non-lethal means of repeated sampling that would not adversely affect the sampled population. Based on the study with chickens, residue analyses of CAMs may not give the best information on more transient OC pesticide levels in birds. Therefore, sampling blood will be done to assess avian exposure to less persistent OC pesticides. Sampling CAMs for persistent OCs may be a better option than egg or blood analyses when determination of embryonic exposure to OCs is desired. Plus, CAMs can be sampled non-lethally and non-invasively which is advantageous for sampling threatened and endangered species.

Sample collection will be accomplished with the collaborative effort of currently active researchers doing studies in the Everglades. Brian Mealey of the Miami Museum of Science is actively involved in research with osprey and will assist in sample collection from osprey. Efforts are currently underway to establish a working relationship with researchers studying great white herons.

A maximum of 15 CAM, blood, and inviable egg samples will be taken from each of five great white heron rookeries within the Everglades. A maximum of 30 CAM, blood, and inviable egg samples will be taken from osprey. Three milliliters of blood will be collected from heron and osprey for residue analyses.

STUDY BENEFITS

This study will serve as a pilot study for a larger exposure assessment planned for south Florida. First, it will show which tissue will give the best information for exposure to persistent and non-persistent OCs. Second, the determined variances for the residue levels will indicate appropriate samples sizes necessary to accurately predict mean exposure levels at given sites. Lastly, it will indicate if OCs in the aquatic system biomagnifying within piscivorous birds.

REFERENCES

- Cobb GP, Wood PD (1997a) Polychlorinated biphenyls in eggs and chorioallantoic membranes of American alligators (*Alligator mississippiensis*) from coastal South Carolina. *Env Tox Chem* 7:1456-1462
- Cobb GP, Wood PD (1997b) PCB concentrations in eggs and chorioallantoic membranes of Loggerhead Sea turtle (*Caretta caretta*) from the Cape Romain National Wildlife refuge. *Chemosphere* 34:539-549
- Cobb GP, Norman DM, Miller MW, Brewer LW (1995) Chlorinated contaminants in chorio-allantoic membranes from Great Blue herons at Whidbey Island Naval Air Station. *Chemosphere* 30:151-164
- Cobb GP, Norman DM, Kendall RJ (1994) Organochlorine contaminant assessment in Great Blue herons using traditional and nonlethal monitoring techniques. *Environ Pollut* 83:299-309

General statement of Problem Area

The problem to be examined is a lack of baseline data involving possible metal contamination in Everglades National Park and insufficient data on the levels of these metals in Osprey populations.

Research Purpose

The primary purpose of this research is to create an accurate measurement of metal concentrations in the Osprey population in Everglades National Park. A secondary purpose is to determine if any bioaccumulation of these elements does occur in conjunction with the existing Mercury levels (Mealey personal comm.) in this population.

Research Problem

There are three facets of the research problem: (1) the existing Mercury concentrations within the Osprey population (we have that from the blood); (2) lack of data on other metal concentrations in this species; (3) the suggestion that populations (are they lower?) are lower due to many factors, one of being the possible contamination from metals.

Significance of Study

This study is significant for three reasons. First, since there is a decline (reference) in successful nestling in Osprey populations in Everglades National Park (may be more regional), which may be contributed to many factors, there is a need to rule out the factor of metal contamination in these populations. Secondly, there is a lack of data to rule out this factor, which this study will produce a baseline measurement of metals in this population. Thirdly, this study is significant because it will determine if

there is bioaccumulation of metal concentrations (very hard to determine in a one or two year project) within the Osprey population.

Research Questions and Hypothesis

Question #1: What concentrations ~~do~~ of other metals exist in the Osprey populations?

Question #2: What do these metal concentrations look like compared against known existing Mercury concentrations in the Osprey?

Question #3: Is there a possibility of bioaccumulation in the Osprey population, does this rule out a toxicological factor in contributing to the decline in nestling success? (establishing the presence of other heavy metal in these raptor populations)

Question #4: If there is a high presence of an element, which congener form does it take?

Hypothesis #1: Concentrations of Selenium, Cadmium, Chromium, Arsenic, Lead, and etc., in the feathers and possibly eggs of Everglades Osprey will have varying levels compared to mercury.

Hypothesis #2: If a high level of a metal exists, it will show isomerism similar to that of Mercury isomerism (eg. elemental Mercury vs. Methyl-Mercury).

Hypothesis #3: Distribution mapping of the Metals will show variations across regions vs. no variation across regions.

Methodology

Design and Instrumentation

The research will use a one-way analysis of variance test. The spatial relationship will be displayed using GIS mapping systems. A univariate/multivariate analysis will be run for the design of the experiment.

The survey instrument will consist of non-invasive procedures of collecting feather samples from nesting sites. This will consist of sites inside the borders ~~of~~ of the Everglades National Park. These sites within the park are of regularly monitored nesting couples. The sampling approach for this method will be collecting juvenile and adult feathers for analysis of contaminants. If possible, eggshells of hatched nestlings will be used to further analyze inorganic levels.

The analysis of feather samples is done with acid digestion and/or dry weighting, acid digestive techniques. Quantitative measurements utilize the Inductivity Coupled Plasma Spectroscopy. If sensitivity is an issue, Atomic Fluorescence Spectrophotometer will measure smaller traces of the element.

The data analysis for this research will be conducted by using the computer package SPSS Windows. The statistical procedures for this analysis are, XXXXX.