

Study Plan
for
Avian Injury Study

Year 3 (2008)

Hudson River Natural Resource Damage Assessment

HUDSON RIVER NATURAL RESOURCE TRUSTEES

State of New York
U.S. Department of Commerce
U.S. Department of the Interior

DRAFT
for
PUBLIC REVIEW AND COMMENT

March 17, 2008

Available from:
U.S. Department of Commerce
National Oceanic and Atmospheric Administration
Hudson River NRDA, Lead Administrative Trustee
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Executive Summary

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees – New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior – are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

As part of the NRDA, the Trustees have conducted several investigations focused on birds, including studies on Hudson River tree swallows in 1994-1995, bird egg preliminary investigations in 2002-2003, and avian injury investigations by the U.S. Geological Survey in 2004-2005. The Trustees also determined that it was appropriate to conduct an avian egg injection study and began such a study in 2006. Year 1 (2006) avian egg injection work focused on injection of test PCBs and development of injection and incubation protocols for eggs from tree swallow, American kestrel and chicken. Year 2 (2007) work entailed an evaluation of the effects of a PCB mixture relevant to tree swallows from the Upper Hudson River in a controlled egg injection study, an evaluation of the effects of *in situ* PCB exposure in Upper Hudson River hatchling tree swallows, and a pilot study of injection of a PCB mixture into eggs of Eastern bluebirds. Analysis of data from these studies is ongoing.

The work proposed for 2008 entails: (1) continuation of the egg injection studies conducted on tree swallows (*Tachycineta bicolor*), American kestrels (*Falco sparverius*) and Eastern bluebirds (*Sialia sialis*) in 2006 and 2007, and (2) a comparison of endpoints in tree swallow and Eastern bluebird eggs collected at the Patuxent Wildlife Research Center (PWRC) and Upper Hudson River sites, with eggs collected at PWRC being used as natural controls for PCB contamination.

The following endpoints in tree swallow, American kestrel, and Eastern bluebird eggs from PWRC and the Upper Hudson River will be assessed in this study:

- Embryo mortality
- Deformities
- Body and organ weights (heart, liver and bursa)
- Bursa histology
- Heart histology
- Gene expression (microarrays and PCR)
- Oxidative Stress (liver)
- CYP450 enzyme induction (liver)
- Thyroid gland T4 content
- Genetic sex

These endpoints were proposed for assessment in Years 1 and 2 and peer reviewed at that time; thus Year 3 peer review of these same injury endpoints will be limited in scope to new and/or otherwise relevant information regarding them that was not reviewed earlier.

The Trustees are interested in receiving feedback on this Draft Study Plan Amendment from the public, and are thus issuing this Draft Study Plan Amendment for public review and comment, in accordance with the Hudson River NRDA Plan. To facilitate this process, the Trustees are asking the public and the party or parties responsible for the contamination to review this Draft Study Plan Amendment and provide feedback on the proposed approach. Comments should be submitted by April 19, 2008. These comments will help the Trustees plan and conduct an assessment that is scientifically valid and cost effective and that incorporates a broad array of perspectives. To that end, the Trustees request that you carefully consider this Draft Study Plan Amendment and provide any comments you may have to:

CONTACT FOR PUBLIC COMMENTS

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1.0 Background

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees – New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior – are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs (Hudson River Natural Resource Trustees 2002).

The Hudson River and surrounding area support more than 150 species of birds, including waterfowl, wading birds, shorebirds, songbirds, and rare species such as the bald eagle, peregrine falcon, and osprey (Andrle and Carroll, 1988). Birds are an integral part of the ecosystem and provide a number of important ecosystem services such as seed distribution, plant pollination, and insect control. Birds are also an important source of prey to other species. Birds may be exposed to PCBs through direct ingestion of contaminated water, sediment, and soil. A more important likely exposure pathway is their consumption of food items that contain PCBs derived from the Hudson River and its floodplain. PCB-contaminated food items linked to the river may include fish, amphibians, benthic invertebrates, adult insects that develop from aquatic larvae, plants growing in or near the river, and mammals that forage in the floodplain.

As part of the NRDA, the Trustees have conducted several investigations focused on birds, including studies on Hudson River tree swallows in 1994-1995 (McCarty and Secord 1999a and 1999b, Secord et al. 1999, Stapleton et al. 2001), bird egg preliminary investigations in 2002-2003 (Hudson River Natural Resource Trustees 2004a, 2005a, 2005b), and avian injury investigations by the U.S. Geological Survey in 2004-2005 (Hudson River Natural Resource Trustees 2004b, 2005c).

The Trustees also determined that it was appropriate to conduct an avian egg injection study and began such a study in 2006 pursuant to study plans (Hudson River Natural Resource Trustees 2006a and 2006b) that were, as appropriate pursuant to the Hudson River NRDA Plan (Hudson River Natural Resource Trustees 2002), subject to peer review and public review and comment. Year 1 (2006) avian egg injection work focused on injection of test PCBs and development of injection and incubation protocols for eggs from tree swallow, American kestrel and chicken.

The Trustees determined it was appropriate to conduct a second year of avian egg injection work (Hudson River Natural Resource Trustees 2007). Year 2 (2007) work entailed an evaluation of the effects of a PCB mixture relevant to tree swallows from the Upper Hudson River in a controlled egg injection study, an evaluation of the effects of *in situ* PCB exposure in Upper Hudson River hatchling tree swallows, and a pilot study of injection of a PCB mixture into eggs of Eastern bluebirds (*Sialia sialis*). Analysis of data from the Year 1 and Year 2 studies is ongoing.

The work proposed for 2008 entails: (1) continuation of the egg injection studies conducted on tree swallows (*Tachycineta bicolor*), American kestrels (*Falco sparverius*) and Eastern bluebirds (*Sialia sialis*) in 2006 and 2007, and (2) a comparison of endpoints in tree swallow and Eastern bluebird eggs collected at the Patuxent Wildlife Research Center (PWRC) and Upper Hudson River sites, with eggs collected at PWRC being used as natural controls for PCB contamination.

These endpoints were proposed for assessment in Years 1 and 2 and peer reviewed at that time, thus Year 3 peer review of these same injury endpoints will be limited in scope to new and/or otherwise relevant information regarding them that was not reviewed earlier.

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2.0 Introduction

This draft Study Plan is for Year 3 (2008) of an avian egg injection and field study.

The primary aim of this study is to integrate data collected in both the lab and field to gain a better appreciation of the impact of PCBs on free-living avian species. This study plan will be carried out in two parts, the first is the *in ovo* lethality aspect, which will be a continuation of the injection studies conducted on tree swallow (*Tachycineta bicolor*), American kestrel (*Falco sparverius*) and Eastern bluebird (*Sialia sialis*) eggs in 2006 and 2007 (Hudson River Natural Resource Trustees 2006b, 2007). The second part is a comparison of endpoints in tree swallow and Eastern bluebird eggs collected at the Patuxent Wildlife Research Center (PWRC) and Upper Hudson River sites, with eggs collected at PWRC being used as natural controls for PCB contamination.

Specific endpoints to be addressed in the study are discussed in section 4.3. Egg injection and collection studies for all species will use the same endpoints.

3.0 Purpose and Objective

The Trustees will conduct a laboratory and field study of tree swallows and Eastern bluebirds in 2008 to evaluate whether specific avian species in the vicinity of the Hudson River are injured due to exposure to PCBs.

This study will be used to evaluate whether the viability of avian resources is affected as a result of exposure to PCBs from the Hudson River. The work will inform the Trustees regarding injury to avian resources and guide their future efforts to identify pathway and specific injuries to birds from PCBs, determine causation, and scale restoration, as defined in the DOI NRDA Regulations. The work will be used to identify and evaluate the type(s) of injury(ies), if any, that PCBs are causing to Hudson River birds. This work will also be used to help determine whether future studies will be performed, and if so, to help in their design.

4.0 Methods

The Trustees have developed the preliminary design described below for work in 2008 to evaluate the effects of exposure of tree swallows to PCBs, through exposure via avian egg injection or through environmental exposure in the field.

4.1 Egg Injection Study with Tree Swallow, American Kestrel and Eastern Bluebird Eggs from PWRC

Tree swallow, American kestrel and Eastern bluebird eggs at PWRC will be injected *in situ* with a mixture of PCB congeners that mimics the spectrum of congeners found in avian eggs in the Upper Hudson River. The eggs will be naturally incubated for at least the first two-thirds of incubation by the parents. This should provide excellent hatching success when eggs are brought to the lab for artificial incubation in the last one-third of incubation. These data will provide a median lethal dose for the field levels of PCB congeners found in tree swallow, American kestrel and Eastern bluebird eggs and allow assessment of the consequences of exposure with natural incubation to optimize embryo survival and mimic natural incubation conditions. Samples from tree swallow, American kestrel and Eastern bluebird eggs will be collected for assessment of the potential endpoints identified in Section 4.3.

4.2 Collection and Assessment of Tree Swallow and Eastern Bluebird Eggs from PWRC and the Upper Hudson River

Tree swallow and eastern bluebird eggs will be collected from the PWRC and Upper Hudson River for analysis to determine if there are differences in the eggs between the two sites that can be attributed to PCB contamination. In this instance there will be no experimental manipulations of the eggs -- endpoints will relate directly to environmental conditions. Samples from tree swallow and Eastern bluebird eggs will be collected for assessment of the potential endpoints identified in Section 4.3.

4.3 Endpoints

The following endpoints in tree swallow, American kestrel and Eastern bluebird eggs from PWRC and the Upper Hudson River will be assessed in this study:

- Embryo mortality
- Deformities

- Body and organ weights (heart, liver and bursa)
- Bursa histology
- Heart histology
- Gene expression (microarrays and PCR)
- Oxidative stress (liver)
- CYP450 enzyme induction (liver)
- Thyroid gland T4 content
- Genetic sex

These endpoints are described in greater detail in the Final Avian Injury Study Plan for 2007 (Hudson River Natural Resource Trustees 2007) and the Final Study Plan for an Avian Egg Injection Study for 2006 (Hudson River Natural Resource Trustees 2006b).

Eggs may also be analyzed for chemical analytes that may include congener-specific PCBs, including the non-*ortho* congeners, polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides, and metals, as determined appropriate by the Trustees.

4.4 Statistical Analyses

Data will be analyzed following examination of normality and proceeding with parametric ANOVAs or non-parametric tests, and regressions as appropriate. When necessary, further analyses may be used to understand the significance of sham or vehicle injection treatment. If the predictions warrant the use of one-tailed tests, these tests will be used with consultation with our statistician. Additional tests may include bootstrap techniques if data are not normally distributed.

Statistical analyses are described in greater detail in the Final Avian Injury Study Plan for 2007 (Hudson River Natural Resource Trustees 2007) and the Final Study Plan for an Avian Egg Injection Study for 2006 (Hudson River Natural Resource Trustees 2006b).

5.0 Quality Assurance/Quality Control

This study is being conducted in accordance with the Quality Assurance Management Plan for the Hudson River NRDA (Hudson River Natural Resources Trustees, 2005d).

Strict chain-of-custody procedures will be used throughout the study. All samples collected under this Study Plan will be maintained under chain-of-custody upon collection, and through processing, storage and shipment to the testing laboratory, analytical laboratory or archive facility.

Analysis will be by appropriate methods approved by the Trustees. As noted above, chemical analytes may include congener-specific PCBs, including the non-*ortho* congeners, polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides, and metals, as determined appropriate by the Trustees.

In order to minimize analytical costs, and reduce the overall cost associated with the project, the Trustees may conduct the chemical or other analyses in stages, using initial work to inform subsequent decisions regarding which analyses to conduct on which samples.

The laboratories performing analytical work will be contracted to follow the Trustees' Analytical Quality Assurance Plan for the Hudson River NRDA (Hudson River Natural Resource Trustees 2005d). Laboratories will provide fully documented data packages which will enable data validation to be performed based on the criteria provided in the Analytical Quality Assurance Plan for the Hudson River NRDA, applicable laboratory Standard Operating Procedures, and the U.S. Environmental Protection Agency guidelines (1999).

Quality assurance/quality control are described in greater detail in the Final Avian Injury Study Plan for 2007 (Hudson River Natural Resource Trustees 2007) and in the Final Study Plan for an Avian Egg Injection Study for 2006 (Hudson River Natural Resource Trustees 2006b).

6.0 Special Provisions

All collection of eggs and any tissues, as well as bird handling, will be conducted under permits from USFWS and appropriate State agencies, and according to appropriate Animal Care and Use Committee approved protocols.

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