

Study Plan
for
Avian Injury Study

Amendment for Year 2 (2007)

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

February 28, 2007

Available from:
U.S. Department of Commerce
National Oceanic and Atmospheric Administration
Hudson River NRDA, Lead Administrative Trustee
Damage Assessment Center, N/ORR31
1305 East-West Highway, Rm 10219
Silver Spring, MD 20910-3281

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. The 2006 Avian Egg Injection Study Plan noted that these studies were projected to continue into a second year, and that work in Year 2 (2007) would be conducted pursuant to a Study Plan Amendment. This document constitutes that proposed Study Plan Amendment.

The work proposed for 2007 entails: (1) evaluating the effects of a PCB mixture relevant to tree swallows from the Upper Hudson River in a controlled egg injection study and (2) evaluating the effects of *in situ* PCB exposure in the Upper Hudson River tree swallows post-hatch by studying the physiology of nestlings, adults and additional hatchlings by comparing measures across a spectrum of PCB exposed birds and between the Upper Hudson River site and reference sites. As Year 2 of the avian egg injection study will entail injury endpoints, the Trustees will perform a peer review of the proposed work and are also issuing this Draft Study Plan Amendment for public review and comment, in accordance with the Hudson River NRDA Plan.

The Trustees are interested in receiving feedback on this Draft Study Plan Amendment. 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 March 30, 2007. 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

Ms. Kathryn Jahn
U.S. Fish and Wildlife Service
3817 Luker Road
Cortland, NY 13045
607-753-9334
kathryn_jahn@fws.gov

Table of Contents

1.0	BACKGROUND	1
2.0	INTRODUCTION.....	2
3.0	PURPOSE AND OBJECTIVE	2
4.0	METHODS	3
4.1	EGG INJECTION STUDY WITH TREE SWALLOW EGGS FROM PATUXENT NWR	3
4.2	UPPER HUDSON RIVER FIELD STUDY OF TREE SWALLOWS.....	4
5.0	QUALITY ASSURANCE/QUALITY CONTROL	5
6.0	SPECIAL PROVISIONS	6
7.0	LITERATURE CITED	6

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 2006 Avian Egg Injection Study Plan noted that these studies were projected to continue into a second year, and that work in Year 2 (2007) would be conducted pursuant to a Study Plan Amendment. This document constitutes that proposed Study Plan Amendment.

As Year 2 of the avian egg injection study will entail injury endpoints, the Trustees will perform a peer review of the proposed work and are also issuing this Draft Study Plan Amendment for public review and comment, in accordance with the Hudson River NRDA Plan.

The Trustees are interested in receiving feedback on this Draft Study Plan Amendment. 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 March 30, 2007. 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

Ms. Kathryn Jahn
U.S. Fish and Wildlife Service
3817 Luker Road
Cortland, NY 13045
607-753-9334
kathryn_jahn@fws.gov

2.0 Introduction

This draft Study Plan Amendment is for Year 2 (2007) of an avian egg injection study.

The work presented here is a continuation of experiments with tree swallows (*Tachycineta bicolor*) initiated in 2006 (Hudson River Natural Resource Trustees 2006b). Previously tree swallow eggs were collected from two sites: Patuxent National Wildlife Refuge (NWR), Maryland and Great Sacandaga Lake, New York. These eggs were injected with PCB 126 in two separate experiments and incubated artificially. Mortality and hatchability of the embryos were monitored and median lethal doses were estimated. The embryos (Patuxent NWR) or hatchlings (Great Sacandaga Lake) were dissected and tissues are being analyzed for a variety of histological and biochemical endpoints. In addition tree swallow eggs naturally exposed to PCBs in the Upper Hudson River were collected mid-incubation and artificially incubated for the remainder of development. After hatch, chicks were dissected and tissues are being analyzed for a variety of histological and biochemical endpoints.

The next steps will be to: (1) evaluate the effects of a PCB mixture relevant to tree swallows from the Upper Hudson River in a controlled egg injection study and (2) evaluate the effects of *in situ* PCB exposure in the Upper Hudson River tree swallows post-hatch by studying the physiology of nestlings, adults and additional hatchlings by comparing measures across a spectrum of PCB exposed birds and between the Upper Hudson River site and reference sites.

3.0 Purpose and Objective

The Trustees will conduct a laboratory and field study of tree swallows in 2007 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 2007 to evaluate the effects of exposure of tree swallows to PCBs, through exposure via avian egg injection or through environmental exposure in the field. There are two components to the work: an egg injection study to be conducted using tree swallow eggs from Patuxent NWR (Maryland) and an Upper Hudson River (New York) field study of tree swallows.

4.1 Egg Injection Study with Tree Swallow Eggs from Patuxent NWR

Tree swallow eggs at Patuxent NWR will be injected *in situ* with a mixture of PCB congeners that mimics the spectrum of congeners found in tree swallow eggs in the Upper Hudson River. The eggs will be naturally incubated for 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 eggs and allow assessment of the consequences of exposure with natural incubation to optimize embryo survival and mimic natural incubation conditions.

The following endpoints will be assessed in tree swallow hatchlings:

Lethality

- Embryo Mortality
- Deformities

Immune

- Immune Structure
 - Bursa Weight and Histology
 - Leukocyte Counts
- Immune Function
 - Plasma Proteins

Reproductive

- Steroid Hormones: estradiol, androgen (fecal and/or plasma)
- Hypothalamic Gonadotropin Releasing-Hormone (GnRH)-I, norepinephrine, dopamine
- Gonad Histology

Metabolic

- Corticosterone (fecal)
- Heart Weight & Histology
- Thyroid Weight & Thyroxine Reserve
- Liver
 - Weight
 - Vitamin A Reserve
 - Histology

- Oxidative Damage
- Supporting Measures
- Body Weight
 - Gender Genotyping
 - RNA Expression

4.2 Upper Hudson River Field Study of Tree Swallows

The second portion of work in 2007 will continue work started in tree swallow hatchlings from the Upper Hudson River in 2006. In 2006 eggs were collected from nests for contaminant analysis and for collection of tissues from hatchlings for a variety of histological and biochemical analyses. In 2007 this work will be continued by analysis of immune function and tissue biochemistry or histology of nestlings before fledging. Both sibling eggs and nestling carcasses from the Upper Hudson River will be analyzed for contaminant levels to determine whether *in ovo* or post-hatch feeding exposure of PCBs, or both, are driving any changes in physiology that might be detected in these birds. Furthermore, data for nestlings at these sites can be compared to data in embryos from the same sites to determine if any adverse effects observed in embryos are also observed in nestlings that have been exposed to PCBs both *in ovo* and post-hatch.

In addition, immune function will be measured in adult tree swallows during rearing of their offspring. Endpoints in nestlings and adults will be compared both (1) within birds exposed to a broad spectrum of PCB concentrations at the Upper Hudson River site and (2) between the Upper Hudson River site and reference sites. Known age adults (banded in 2006) will have been exposed to PCBs at the Upper Hudson River *in ovo*, as nestlings and during breeding.

This study will enhance our characterization of the impact of environmentally relevant concentrations of PCBs in the area of interest. In addition, some hatchlings may be sampled at the Upper Hudson River sites to increase the sample size from 2006. Results will be compared across a spectrum of PCB exposed birds and between the Upper Hudson River site and hatchlings from untreated eggs collected at Patuxent NWR as part of the egg injection study.

The following endpoints will be assessed in tree swallow nestlings:

Immune

- Immune Structure
 - Bursa Weight and Histology
 - Leukocyte Counts
- Immune Function
 - PHA Skin Response
 - Plasma Proteins

Reproductive

- Steroid Hormones: estradiol, androgen (fecal &/or plasma)
- Hypothalamic GnRH-I, norepinephrine, dopamine
- Gonad Histology

Metabolic

- Corticosterone (fecal &/or plasma)

- Heart Weight & Histology
- Thyroid Weight & Thyroxine Reserve
- Liver
 - Weight
 - Vitamin A Reserve
 - Histology
 - Oxidative Damage

Supporting Measures

- Body Weight
- Gender Genotyping
- RNA Expression

The following endpoints will be assessed in tree swallow adults:

Immune Structure and Function

- Immune Structure
 - Leukocyte Counts
- Immune Function
 - PHA Skin Response
 - Plasma Proteins
 - Antibody Response

Supporting Measures

- Body Weight
- Gender Genotyping
- Corticosterone (plasma)

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. 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).

6.0 Special Provisions

Any necessary collection permits, such as those from New York State or Maryland where eggs will be collected, or from the U.S. Fish and Wildlife Service, will be obtained.

7.0 Literature Cited

Andrle, R.F. and J.R. Carroll. 1988. *The Atlas of Breeding Birds in New York State*. Cornell Univ. Press. 548 pp.

Hudson River Natural Resource Trustees. 2002. Hudson River Natural Resource Damage Assessment Plan. September 2002. U.S. Department of Commerce, Silver Spring, MD.

Hudson River Natural Resource Trustees. 2004a. Work Summary and Data Report for the Collection of Eggs from American Peregrine Falcon, Hudson River, New York. Hudson River Natural Resource Damage Assessment. Final Report. December 24, 2004. U.S. Department of Commerce, Silver Spring, MD.

Hudson River Natural Resource Trustees. 2004b. Study Plan for Year 2004 Avian Investigations for the Hudson River. Final. Public Release Version. June 15, 2004. U.S. Department of Commerce, Silver Spring, MD.

Hudson River Natural Resource Trustees. 2005a. Data Report for the Collection of Eggs from Spotted Sandpipers, American Woodcock, Belted Kingfisher, American Robin, Red-Winged Blackbird, and Eastern Phoebe Associated with the Hudson River from Hudson Falls to Schodack Island, New York. Hudson River Natural Resource Damage Assessment. Final. Report Released September 17, 2004, Revised June 15, 2005. U.S. Department of Commerce, Silver Spring, MD.

Hudson River Natural Resource Trustees. 2005b. Data Report for the Collection of Eggs from Eastern Screech Owl Associated with the Hudson River from Hudson Falls to Schodack Island, New York. Hudson River Natural Resource Damage Assessment. Final Report. April 28, 2005. U.S. Department of Commerce, Silver Spring, MD.

Hudson River Natural Resource Trustees. 2005c. Modification to Study Plan for Avian Investigations for the Hudson River. USGS Study Plan Amendment for 2005. Final. Public Release Version. May 4, 2005. U.S. Department of Commerce, Silver Spring, MD.

- Hudson River Natural Resource Trustees. 2005d. Analytical Quality Assurance Plan for the Hudson River Natural Resource Damage Assessment. Final. Public Release Version. September 1, 2005. Version 2.0. U.S. Department of Commerce, Silver Spring, MD.
- Hudson River Natural Resource Trustees. 2006a. Modification to Study Plan for Avian Investigations for the Hudson River. USGS Study Plan Amendment for 2006. Final. Public Release Version. May 9, 2006. U.S. Department of Commerce, Silver Spring, MD.
- Hudson River Natural Resource Trustees. 2006b. Study Plan for Avian Egg Injection Study. Hudson River Natural Resource Damage Assessment. Final. Public Release Version. May 12, 2006. U.S. Department of Commerce, Silver Spring, MD.
- McCarty, J.P. and A.L. Secord. 1999a. Reproductive Ecology of Tree Swallows (*Tachycineta bicolor*) With High Levels of Polychlorinated Biphenyl Contamination. Environ. Toxicol. Chem. 18:1433-1439.
- McCarty, J.P. and A.L. Secord. 1999b. Nest-Building Behavior in PCB-Contaminated Tree Swallows. The Auk 116:55-63.
- Secord, A.L., J.P. McCarty, K.R. Echols, J.C. Meadows, R.W. Gale, and D.E. Tillit. 1999. Polychlorinated Biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in tree swallows from the Upper Hudson River, New York State, USA. Environ. Toxicol. Chem. 18:2519-2525.
- Stapleton, M., P.O. Dunn, J. McCarty, A. Secord, and L.A. Whittingham. 2001. Polychlorinated biphenyl contamination and minisatellite DNA mutation rates of tree swallows. Environ. Toxicol. Chem. 20:2263-2267.
- U.S. Environmental Protection Agency. 1999. USEPA Contract Laboratory Program National Functional Guidelines for Organic Data Review. Office of Emergency and Remedial Response, Washington, D.C. 20460. EPA540/R-99/008, 118 pp.