# STUDY PLAN FOR AVIAN EGG INJECTION STUDY

# 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

## PUBLIC RELEASE VERSION*

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**Names of certain individuals and affiliations have been removed to maintain confidentiality*

REVISION HISTORY
to

Study Plan for Avian Egg Injection Study

The following changes have been made since the original release of this document on May 12, 2006:

1. Appendix A was revised as described therein on p. 2 of 87.

2. Appendix B was replaced in its entirety.

3. All references to the “Final Work Plan for Tree Swallow, American Kestrel, and Chicken Egg Injection Studies” have been changed to the “Revised Work Plan for Tree Swallow, American Kestrel, and Chicken Egg Injection Study.”

## 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.

In 2002, the Trustees conducted an avian egg exposure preliminary investigation for the Hudson River. That preliminary investigation revealed that of the eleven avian species tested, the highest PCB levels were found in belted kingfisher and spotted sandpiper.

Based on the results of avian investigations conducted by the Trustees, and considering factors such as the life histories of various Hudson River avian species, avian toxicology, and the goals of the NRDA, the Trustees determined that it was appropriate to conduct further investigations focused on avian species. Pursuant to that determination and to the Hudson River NRDA Plan, the Trustees conducted a study of belted kingfisher, spotted sandpiper and tree swallow in 2004 and 2005. The Trustees further proposed conducting an avian egg injection study.

A Draft Study Plan for the egg injection work was peer reviewed and made available to the public for review and comment. All comments received on the Draft Study Plan, as part of the peer and public review process, have been considered. The Trustees evaluated peer and public comments and, where warranted, incorporated these comments in the Draft Study Plan to produce the Final Study Plan. In the remaining instances, public comments on the Draft Study Plan have been addressed by letters to the commenters, acknowledging receipt of comments and providing an initial response and noting that a more detailed Responsiveness Summary will be provided by the Trustees in the near future.

The Trustees will conduct an avian egg injection study of tree swallow and American kestrel in 2006 to evaluate whether specific avian species in the vicinity of the Hudson River are injured due to exposure to PCBs. Additionally, egg injection experiments will be conducted using chicken eggs to provide a point of reference for impacts observed in other species and in relation to effects levels identified in the toxicology literature for PCBs, dioxins, and other chemicals. Work on the species studied in 2006 or other species may continue in 2007.

The objective of the investigation is to evaluate the toxicity and adverse effects of embryonic exposure of multiple avian species to dose ranges of PCB 126 or a PCB mixture. This study will be used to evaluate whether avian reproduction and/or development is affected as a result of exposure to PCBs from the Hudson River.

Pursuant to the Hudson River NRDA Plan, the results of the work conducted pursuant to this Study Plan will be peer reviewed upon completion of the study, and the results then released to the public.

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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.

In 2002, the Trustees conducted an avian egg exposure preliminary investigation for the Hudson River. The investigation entailed collection of eggs, and subsequent analysis for PCBs, from six primary species (belted kingfisher (*Ceryle alcyon*), American robin (*Turdus migratorius*), Eastern phoebe (*Sayornis phoebe*), spotted sandpiper (*Actitis macularia*), red winged blackbird (*Agelaius phoenicius*), and American woodcock (*Scolopax minor*)) and from five additional species (Eastern screech owl (*Otus asio*), common grackle (*Quiscalus quiscula*), northern rough-winged swallow (*Stelgidopteryx serripennis*), barn swallow (*Hirundo rustica*), and Eastern bluebird (*Sialia sialis*)) based on the opportunities for survey team members to locate the nests of these species. The geographic scope of the 2002 avian egg investigation was the Hudson River and its floodplains, from Hudson Falls to Lower Schodack Island, New York.

That preliminary investigation was undertaken by the Trustees to assist in determining the extent to which avian species in the Hudson River are contaminated with PCBs, to determine if additional pathway and injury assessment studies focused on avian species should be conducted as part of the Hudson River NRDA, and for potential use in the design of future studies to assess the health of Hudson River birds. The Trustees noted in the Hudson River NRDA Plan that, based on the results of the bird egg study, the Trustees would determine whether injury determination and quantification studies were warranted.

That preliminary investigation revealed that of the eleven avian species tested, the highest PCB levels were found in belted kingfisher and spotted sandpiper (Hudson River Natural Resource Trustees, 2003). Spotted sandpiper eggs contained a mean of 15 parts per million (ppm) PCBs (as total homologues, fresh weight basis). Of the eleven species tested, spotted sandpiper eggs exhibited the highest individual egg concentration of PCBs (56 ppm) as well as the highest average PCB concentration (15 ppm). Of the eleven species tested, belted kingfisher eggs exhibited the second highest individual egg concentration of PCBs (43 ppm).

Based on the results of avian investigations conducted by the Trustees, including the tree swallow (*Tachycineta bicolor*) work (McCarty and Secord 1999a, 1999b, Secord *et al.* 1999) and the 2002 avian egg preliminary investigation (Hudson River Natural Resource Trustees 2003), and input from a panel of avian experts, and considering factors such as the life histories of various Hudson River avian species, avian toxicology, and the goals of the NRDA, the Trustees determined that it was appropriate to conduct further investigations focused on avian species, and initially on belted kingfisher, spotted sandpiper, and tree swallow, to be started in the year 2004.

Pursuant to that determination and to the Hudson River NRDA Plan, the Trustees released in 2004 a "Study Plan for Year 2004 Avian Investigations for the Hudson River - Final, Public Release Version," dated June 15, 2004 (Hudson River Natural Resource Trustees, 2004). That Avian Injury Study Plan described the activities that constituted the Trustees' planned approach to conducting investigations of injury to avian species as part of the Hudson River NRDA.

As noted in the Avian Injury Study Plan, the Trustees planned to assess the following potential injuries to birds: reduced avian reproduction and overt external malformations. The Trustees planned to: (1) assess the relationship between contaminant concentrations in nest sample eggs and parameters of nest reproduction by application of appropriate statistical analysis of data to determine whether reproductive success of spotted sandpipers, tree swallows and belted kingfishers nesting on the Hudson River is negatively affected by PCB exposure; (2) assess the incidence of gross deformities in embryos or hatchlings; (3) assess organic contaminant accumulation rates in belted kingfisher chicks on the Hudson River; and (4) initiate an avian egg injection pilot study in 2004.

Pursuant to the Final Avian Injury Study Plan and a May 4, 2005 Modification to that Study Plan (Hudson River Natural Resource Trustees, 2005a), the U.S. Geological Survey (USGS) conducted a study of belted kingfisher, spotted sandpiper and tree swallow in 2004 and 2005. The USGS study was directed at items (1), (2) and (3) above. Trustee review of the data and results from the USGS study is ongoing.

Regarding item (4) above, the Trustees' Final Avian Injury Study Plan proposed a "pilot" study, a preliminary investigation focused on incubation of eggs of Hudson River avian species in 2004 with injection of PCBs into eggs of avian species of interest in a subsequent year. The Trustees subsequently determined that, considering preliminary work done by the Trustees and the literature on avian egg injection studies, it was not necessary to conduct a separate incubation-focused pilot study prior to initiating an avian egg injection study.

A Draft Study Plan for an avian egg injection experiment (Hudson River Natural Resource Trustees 2006) was developed, and that Draft Study Plan was peer reviewed and made available to the public for review and comment. All comments received on the Draft Study Plan, as part of the peer and public review process, have been considered. The Trustees evaluated peer and public comments and, where warranted, incorporated these comments in the Draft Study Plan to produce a Final Study Plan. In the remaining instances, public comments on the Draft Study Plan have been addressed by letters to the commenters, acknowledging receipt of comments and providing an initial response and noting that a more detailed Responsiveness Summary will be provided by the Trustees in the near future.

## 2.0 INTRODUCTION

Avian egg injection is a well-established technique to assess the effects of contaminants on a developing avian embryo (Allred and Strange 1977, Blankenship et al. 2003, Blomqvist et al. 2006, Boily et al. 2003, Bruggeman et al. 2003, Brunström 1986, Brunström et al. 1999, Brunström 1990, Brunström 1988, Brunström and Andersson 1988, de Roode et al. 2000, DeWitt et al. 2005a, DeWitt et al. 2005b, Drake et al. 2006, Fernie et al. 2005, Fox and Grasman 1999, Goff et al. 2005, Gould et al. 1997, Grasman and Whitacre 2001, Halldin et al. 2002, Heinz et al. 2006, Hoffman et al. 1998, Ivnitski et al. 2001, Janz and Bellward 1996, Jin et al. 2001, Katynski et al. 2004, Lim et al. 2005, Meneely and Wuttenbach 1989, Murvoll and Skaare 2005, Nosek et al. 1993, Ottinger et al. 2005, Powell et al. 1996a, Powell et al. 1996b, Powell et al. 1997a, Powell et al. 1997b, Powell et al. 1998, Stanton et al. 2003, U.S. Environmental Protection Agency 2001, Walker and Catron 2000, Walker et al. 1997, Wilhelms et al. 2006, Zhang et al. 2002, and Zhao et al. 1997).

To conduct an avian egg injection experiment, eggs are collected and brought into a laboratory where they are injected with the substance being tested. In avian egg injection experiments, various doses of a contaminant of concern (for example, PCBs in a vehicle or carrier solution) are typically injected into the yolk sac (for example, Hoffman et al. 1998), air cell (for example, Brunström and Andersson 1988, Fox and Grasman 1999), or albumen (for example, Nosek et al. 1993) of eggs. The eggs are then incubated in a laboratory and their development monitored. Measurement endpoints may include embryomortality, malformations, and hatching success. Measurement endpoints may also extend to hatchlings, for which chick growth and development, for example, may be measured.

Results reported in the literature of injecting contaminants, such as PCBs, into avian eggs include embryomortality and malformation. Death, including embryomortality, for example, and physical deformation, such as external malformation, skeletal deformities, and organ and soft tissue malformation, are injuries pursuant to the regulations written by the U.S. Department of the Interior contained in Title 43 of the Code of Federal Regulations Part 11, Natural Resource Damage Assessment (the "DOI NRDA Regulations"), and would be relevant to determining injury as part of the NRDA.

## 3.0 PURPOSE AND OBJECTIVE

The Trustees will conduct an avian egg injection study of tree swallow and American kestrel in 2006 to evaluate whether specific avian species in the vicinity of the Hudson River are injured due to exposure to PCBs. Additionally, egg injection experiments will be conducted using chicken eggs to provide a point of reference for impacts observed in other species and in relation to effects levels identified in the toxicology literature for PCBs, dioxins, and other chemicals. Work on the species studied in 2006 or other species may continue in 2007.

The objective of the investigation is to evaluate the toxicity and adverse effects of embryonic exposure of multiple avian species to dose ranges of PCB 126 or a PCB mixture. The PCB mixture is made up of individual PCB congeners and fits a similar profile to the mixture of PCBs occurring in the eggs of birds nesting in the Upper Hudson River. This study will be used to evaluate whether avian reproduction and/or development 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 at the embryonic level. 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

## *4.1 YEAR 1 (2006) AVIAN EGG INJECTION STUDY OF TREE SWALLOW, AMERICAN KESTREL, AND CHICKEN*

The attached work plan entitled, "Revised Work Plan for Tree Swallow, American Kestrel, and Chicken Egg Injection Studies" (Appendix A) describes the avian egg injection investigation that the Trustees will implement to evaluate whether specific avian species in the vicinity of the Hudson River are injured due to exposure to PCBs. The attached work plan includes information regarding the experimental design, Quality Assurance/Quality Control, and Standard Operating Procedures that will be used in the study.

In Year 1 (2006), the Trustees will focus on injection of test PCBs and incubation methods for eggs from chosen species. If injection and incubation methods are successful, tissues will be collected for analysis, providing initial datasets for these species. Year 1 (2006) work will focus on those species with eggs that are more easily obtainable than others.

In 2006, work will be conducted on tree swallow and American kestrel (*Falco sparverius*). These species have been selected because they represent different positions in the ecosystem, breed in the Hudson River basin, and may be sensitive to PCB exposure. Eggs of American kestrel will be obtained from Patuxent Wildlife Research Center, Maryland. Eggs of tree swallows will be obtained from Patuxent National Wildlife Refuge, Maryland, from a breeding colony on Great Sacandaga Lake, New York, and from the Hudson River, New York. Additionally, egg injection experiments will be conducted using chicken (*Gallus domesticus*) eggs to provide a point of reference for impacts observed in other species and in relation to effects levels identified in the toxicology literature for PCBs, dioxins and other chemicals. Work will begin in Spring 2006.

Trials will be conducted using select PCBs administered early in embryonic development. As described in the "Revised Work Plan for Tree Swallow, American Kestrel, and Chicken Egg Injection Studies," eggs will be injected with PCBs (PCB 126 or a PCB mixture reflective of chemical exposures in the Hudson River region). A vehicle control and an untreated group of eggs will be included. The PCBs to be injected into the eggs have been selected by the Trustees based on existing contaminants data from Hudson River biota and other relevant factors. Appendix B provides information on the PCB congener mixture to be used in the egg injections.

There will be a separate experiment conducted for each species. The timing of each experiment will depend on the availability of eggs for that species. In the case of chickens, for example, eggs will be available over much of the year, so those experiments will be scheduled around the work with the other species.

The "Revised Work Plan for Tree Swallow, American Kestrel, and Chicken Egg Injection Studies" (Appendix A) notes the endpoints to be assessed and the methods that will be used.

## *4.2 YEAR 2 (2007) AVIAN EGG INJECTION STUDY*

Year 1 work focuses on injection of test PCBs and incubation methods for eggs from tree swallow, American kestrel and chicken. These studies are projected to continue into a second year to allow further development of injection and incubation protocols for eggs from wild species and, in some cases, to produce larger sample sizes. Work on the species from 2006 or other species may be conducted in 2007. Work in 2007 will be conducted pursuant to a Study Plan Amendment for Year 2007.

## 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, 2005b).

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 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 2005b). 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.

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