Study Plan
For Year 2004 Avian Investigations
for the Hudson River

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

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Damage Assessment Center, N/ORR31
1305 East-West Highway, Rm 10219
Silver Spring, MD 20910-3281

Executive Summary

Natural resources of the Hudson River have been contaminated through past and ongoing discharges of polychlorinated biphenyls (PCBs). 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 a means of evaluating regional avian contamination, a screening level survey of PCB levels in avian eggs was conducted from April – June 2002. That investigation revealed that of the eleven avian species studied, eggs from spotted sandpiper (*Actitis macularia*) and belted kingfishers (*Ceryle alcyon*) exhibited the highest levels of PCB contamination (on a total homologue basis, fresh-weight adjusted). Tree swallows from the Hudson River are also known to be contaminated with PCBs, based on earlier work by the Trustees.

Based on the results of avian investigations conducted by the Trustees, including the tree swallow work and the 2002 avian egg preliminary investigation, 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 goal of the NRDA, the Trustees have determined that it is appropriate to conduct further investigations focused on avian species, particularly belted kingfisher, spotted sandpiper, and tree swallow, to be initiated in the year 2004.

Pursuant to the Hudson River NRDA Plan, the Trustees have developed this Study Plan for an avian injury determination effort. This Study Plan describes the activities that constitute the Trustees' currently proposed approach to conducting investigations of avian species, particularly belted kingfisher, spotted sandpiper, and tree swallow, beginning in Spring 2004, as part of the Hudson River NRDA. This study plan will be peer reviewed and is being made available to the public for review and comment.

The Trustees propose to assess the following potential injuries to these birds: reduced avian reproduction and overt external malformations. The Trustees propose to assess the relationship between contaminant concentrations in nest sample eggs and parameters of nest reproduction by application of appropriate statistical analysis of data, and to determine whether reproductive success of spotted sandpipers, tree swallows and belted kingfishers nesting on the Hudson River is negatively impacted by PCB exposure. The Trustees will also assess the incidence of gross deformities in embryos or hatchlings, assess organic contaminant accumulation rates in belted kingfisher chicks on the Hudson River; and assess monooxygenase activity in belted kingfisher chicks on the Hudson River. The Trustees also plan to initiate an avian egg injection pilot study in 2004.

The purpose of this work is to inform the Trustees regarding injury to avian resources and guide their future efforts to identify pathway and specific injuries to birds from PCBs, as defined in 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. This work will be used to help determine whether future studies will be performed in the year 2005 and potentially beyond, and if so, to help in their design.

The Trustees are interested in receiving feedback on this Draft Study 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 and provide feedback on the proposed approach. Comments should be submitted by April 15, 2004 to:

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

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 2002a).

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.

Tree swallows from the Hudson River are known to be contaminated with PCBs. A recent set of studies examined tree swallows (*Tachycineta bicolor*) in the Hudson River basin. Tree swallows nesting along the upper Hudson River had PCB concentrations up to 114 parts per million (ppm) total PCBs based on fresh wet weight of adult whole bodies (Secord *et al.* 1999, Stapleton *et al.* 2001). Tree swallows from the Hudson River have egg PCB concentrations ranging from 9.3 to 29.5 ppm, while concentrations in nestlings ranged from 3.7 to 62.2 ppm (McCarty and Secord 1999a). Reproductive effects observed included supernormal clutch size, reduced hatchability due to failure of embryos to develop (presumably infertile) and death of deformed embryos, high rates of nest abandonment, and other abnormal parental behavior (McCarty and Secord 1999a, 1999b).

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 the Trustees 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 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).

Both spotted sandpipers and belted kingfishers are reported to be relatively common breeders along the Hudson River and its floodplains (Andrle and Carroll 1988). Spotted sandpipers and belted kingfishers consume different types of foods and generally represent different ecological guilds. Spotted sandpipers consume extensive amounts of insects, often from wetland or riparian habitats (Degraaf and Yamasaki 2001, Ehrlich *et al.* 1988). Belted kingfishers primarily consume extensive amounts of small fish but their diet also may include aquatic invertebrates (Ehrlich *et al.* 1988).

In birds, mortality, deformity, and other toxicological effects to embryos and nestlings have been found to be correlated with PCB contamination in eggs; effects vary according to species sensitivity, levels of contamination, and composition of PCB mixtures (Brunstrom and Reutergardh 1986, Brunstrom 1989, Bush et al. 1974, Hill et al. 1975). Known effects of PCBs on birds include decreased hatching success, delayed hatching, increased embryonic deformity rates (e.g., beak and limb deformities, cardiovascular malformation), inhibition of lymphoid development, edema (pericardial and subcutaneous), liver lesions, decreased organ weights, and reduced growth and survival (Barron et al. 1995, Bosveld et al. 1995, Bosveld and Van den Berg 1994, Custer et al. 2003a, Fernie et al. 2001, Gilbertson et al. 1991, Hoffman et al. 1986, Hoffman et al. 1998, Larson et al. 1996, McCarty and Secord 1999a, 1999b, Powell et al. 1998, Yamashita et al. 1993).

2.0 Introduction

Based on the results of avian investigations conducted by the Trustees, including the tree swallow 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 goal of the NRDA, the Trustees have determined that it is appropriate to conduct further investigations focused on avian species, particularly belted kingfisher, spotted sandpiper, and tree swallow, to be initiated in the year 2004.

Pursuant to the Hudson River NRDA Plan, the Trustees have developed this Study Plan for an avian injury determination effort. This Study Plan describes the activities that constitute the Trustees' currently proposed approach to conducting investigations of avian species, particularly belted kingfisher, spotted sandpiper, and tree swallow, beginning in Spring 2004, as part of the Hudson River NRDA.

The Trustees propose to assess the following potential injuries to these birds: reduced avian reproduction and overt external malformations. The Trustees propose to assess the relationship between contaminant concentrations in nest sample eggs and parameters of nest reproduction by application of appropriate statistical analysis of data, and to determine whether reproductive success of spotted sandpipers, tree swallows and belted kingfishers nesting on the Hudson River is negatively impacted by PCB exposure. The Trustees will also assess the incidence of gross deformities in embryos or hatchlings, assess organic contaminant accumulation rates in belted kingfisher chicks on the Hudson River; and assess monooxygenase activity in belted kingfisher chicks on the Hudson River. Additionally, the Trustees will initiate an avian egg injection pilot study in 2004.

The purpose of this work is to inform the Trustees regarding injury to avian resources and guide their future efforts to identify pathway and specific injuries to birds from PCBs, as defined in 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. This work will be used to help determine whether future studies will be performed in the year 2005 and potentially beyond, and if so, to help in their design.

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.

The Trustees are interested in receiving feedback on this Draft Study 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 and provide feedback on the proposed approach. Comments should be submitted by April 15, 2004. These comments will help the Trustees plan and conduct an assessment that is scientifically valid, cost effective, and that incorporates a broad array of perspectives. To that end, the Trustees request that you carefully consider this Draft Study Plan 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

3.0 Purpose

The purpose of this work is to inform the Trustees regarding injury to avian resources and guide their future efforts to identify pathway and specific injuries to birds from PCBs, as defined in 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. This work will be used to help determine whether future studies will be performed in the year 2005 and potentially beyond, and if so, to help in their design.

The Trustees have determined that further investigations focused on avian species, particularly belted kingfisher, spotted sandpiper, and tree swallow, are appropriate to injury assessment for the Hudson River NRDA, and have designed a suite of studies, to be initiated in 2004, with additional follow-up work to be conducted in 2005 and beyond if the results of the work conducted in 2004 indicate that injury exists and further study is warranted.

4.0 Methods

4.1 Geographic Scope of Investigation

This work will focus on the Hudson River between Bakers Falls on the Hudson River (in Hudson Falls, New York) and Lower Schodack Island, New York.

4.2 U.S. Geological Survey Study of Belted Kingfisher, Spotted Sandpiper and Tree Swallow

On behalf of the Trustees, in Spring-Summer 2004, scientists from the U.S. Geological Survey (USGS) will conduct an investigation of Hudson River belted kingfishers, spotted sandpipers and tree swallows with the following three components.

4.2.1 Assessment of reproductive success of belted kingfisher, spotted sandpiper and tree swallow, and assessment of the incidence of gross deformities in embryos or hatchlings of these birds

The following tasks will be accomplished:

- Locate nests of belted kingfishers, tree swallows and spotted sandpipers breeding along the Hudson River from Bakers Falls to Lower Schodack Island, and in a region upstream of Glens Falls, New York. Identify reference area(s) as needed.
- Determine the reproductive success of belted kingfishers, tree swallows and spotted sandpipers at study nests.
- Collect a sample egg (Blus et al. 1974) from each accessible nest of belted kingfisher, spotted sandpiper or tree swallow; belted kingfisher and spotted sandpiper eggs will be analyzed for a suite of contaminants (including PCBs, polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides); tree swallow eggs will be analyzed only if determined warranted by the Trustees;
- In study nests of belted kingfisher or spotted sandpiper where failed eggs are discovered, concentrations of the contaminant suite in all such failed eggs (or in a representative egg from failed clutches) will be determined; where nest failure occurs, the suite of contaminants may be measured in all such eggs of failed clutches. Failed tree swallow eggs (individual eggs, eggs representative of clutches, or complete failed clutches) will be analyzed only if determined warranted by the Trustees; embryos will be examined for gross deformities, where possible.

Nest surveys will focus on the Hudson River from Bakers Falls to Lower Schodack Island, on the reach upstream of Glens Falls as a reference location, and possibly on a neighboring reference river (Connecticut River or others).

Once sandpiper and kingfisher nests are located they will be visited and clutch size, hatching success, and fledging success (kingfishers only) determined. A sample egg will be randomly collected from each nest at an appropriate time to minimize the chance of nest abandonment, and success of the remaining eggs will be monitored. Spotted sandpiper eggs will be monitored to hatching to determine hatching success. Belted kingfisher eggs will be monitored to hatching to determine hatching success, and then to fledging to determine fledging success. For both belted kingfishers and spotted sandpipers, egg weights for collected eggs and incidence of gross deformities in embryos or hatchlings will be documented. Egg contents will be removed in a field laboratory, stored frozen (-20 degrees C), then shipped to the Trustees' analytical laboratory.

Reproductive data will be compiled using Mayfield's estimate of daily egg survival (Mayfield 1961, 1975) and compared among sites using methods outlined in Hensler and Nichols (1981). Contrasts (Sauer and Williams 1989) will be used to make comparisons among sites.

The sample egg technique (Blus et al. 1974) will be used to examine relationships between organic contaminant concentrations in eggs and reproductive success (Custer et al. 2003a, Custer et al. 2003b).

In study nests of belted kingfisher or spotted sandpiper where failed eggs are discovered, concentrations of the contaminant suite in all failed eggs (or in a representative egg from failed clutches) will be determined. Where nest failure occurs, the suite of contaminants will be measured in all eggs of failed clutches of belted kingfisher or spotted sandpiper. Where possible, embryos will be examined for gross deformities.

Based on these activities, the Trustees will assess the relationship between contaminant concentrations in nest sample eggs and parameters of nest reproduction by application of appropriate statistical analysis of data, and determine whether reproductive success of spotted sandpipers, tree swallows and belted kingfishers nesting on the Hudson River is negatively impacted by PCB exposure. The Trustees will also assess the incidence of gross deformities in embryos or hatchlings.

4.2.2 Determination of organic contaminant accumulation rates in belted kingfisher chicks on the Hudson River

The accumulation of contaminant mass (μg/day) in nestlings has been used as an indicator of local contamination (Custer et al. 2003a, Custer et al. 2003b, Secord et al. 1999). Because food fed to nestlings is from areas nearby the nesting location and the calculation of accumulation rate factors out the contribution of contaminant mass from the egg (Ankley et al. 1993), the accumulation of contaminant mass in nestling tissues reflects local contamination.

To accomplish this work, one 10-day-old nestling will be collected from 10 kingfisher broods on the Hudson River and 10 broods from nearby reference locations. Kingfisher nestling age will be based on date of egg laying, the estimated date of hatching, and morphological features. The day of hatching will be considered day 0.

Accumulation rates will be calculated as the mass of the contaminant in the nestling minus the mass of the contaminant in a sibling egg divided by the age of the nestling (Custer et al. 2003a, 2003b).

4.2.3 Determination of monooxygenase activity in belted kingfisher chicks on the Hudson River

PCBs have been reported to increase the content or activity of several enzymes in birds, including P450 isozymes (Hoffman et al., 1996). For example, planar PCBs strongly induce the P450 isozyme CYP1A [measured by increases in aryl hydrocarbon hydroxylase (AHH) or ethoxyresorufin-O-deethylase (EROD) activity]. Each isozyme has a slightly different affinity and capacity for metabolizing contaminants and endogenous biomolecules (e.g., steroids), and may be differentially induced by exposure to PCBs. Numerous studies have reported EROD induction by dioxin-like PCBs, and some have suggested EROD induction as a sensitive measure of exposure. EROD induction may also be indicative of adverse PCB effects because P450 isozymes may activate or deactivate endogenous biomolecules (e.g., hormones). For example, Lincer and Peakall (1970) reported increased microsomal metabolism of estradiol in American kestrels fed a diet containing Aroclor 1254. However, Custer et al. (1997) showed that EROD activity in great-blue heron eggs from the upper Mississippi River was not correlated with concentrations of total PCBs or 2,3,7,8-TCDD. Recent in vitro studies demonstrate that EROD activity in bird tissues is suppressed at higher PCB concentrations (e.g., Lorenzen et al., 1997).

To accomplish this work, immediately after euthanization, the liver will be removed from kingfisher nestlings, weighed, and about 0.3 g from one nestling per brood placed into a cryotube for measurement of monooxygenase activity. A few drops of glycerin are added to the cryotube and the cryotubes will be immediately placed into liquid nitrogen. The cryotubes may be transferred from the liquid nitrogen to an ultracold freezer (-80^{o}C) for storage until assayed. Monooxygenase activities will be assayed at a laboratory selected by the Trustees using appropriate methods such as those of Burke and Mayer (1983) as adapted to a fluorescence microwell plate scanner (Melancon 1996).

4.3 Avian Egg Injection Pilot Study

Egg injection assesses the effects of contaminants on a developing avian embryo. Avian egg injection experiments typically use various doses of a contaminant of concern (for example, PCBs) injected into the egg (injection sites include the yolk sac, air cell, or albumen of the eggs). The eggs are then incubated in a laboratory and monitored. Measurement endpoints may include embryomortality, malformations, hatching success, and chick growth, if hatchlings are monitored.

Eggs of select avian species, including belted kingfisher and spotted sandpiper, will be collected from nests and brought to the laboratory to determine the feasibility of incubation. There will also be analysis of eggs from these sites, or chicks resulting from the incubation, to confirm that these potential sources of eggs for future egg injection studies are relatively uncontaminated. These sources of eggs may be within certain parts of the Hudson River watershed or outside of the watershed. Egg injection protocols may also be investigated, such as selection of doses, the choice of a carrier solution for the contaminants of concern, and the place of injection. Appropriate protocols and quality control/quality assurance plans will be developed by the Trustees prior to beginning the work.

Based on the results of the pilot study initiated in 2004, the Trustees will determine if it is appropriate to pursue further an egg injection study. Potential subsequent work would entail injections of selected contaminants of concern into eggs of avian species of interest, and assessment of endpoints. A Study Plan would be prepared for such subsequent work; such a Plan would be peer reviewed and provided to the public for review and comment.

5.0 Quality Assurance/Quality Control

This study is being conducted in accordance with the Quality Assurance Management Plan for the Trustees' Hudson River NRDA (Hudson River Natural Resources Trustees, 2002b).

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 (including euthanization, if required), storage and shipment to the analytical laboratory or archive facility.

All samples will be analyzed by appropriate methods approved by the Trustees, for analytes including congener specific PCBs, including the non-*ortho* congeners, for PCDDs, PCDFs, PBDEs, and organochlorine pesticides.

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 2002b), which will be updated to include measurement quality objectives for the additional analytes. 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 (1999).

6.0 Special Provisions

Collecting permits will be required from New York State, the U.S. Fish and Wildlife Service, and the National Park Service. Any such necessary permits will be obtained.

The work to be done by USGS scientists will be reviewed and approved by a USGS Animal Care and Use Committee prior to beginning collection.

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