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

PUBLIC RELEASE VERSION*

FINAL

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Silver Spring, MD 20910-3281

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

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 PCB 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 (*Tachycineta bicolor*) 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 goals 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' planned 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.

A Draft Study Plan for this 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 commenter, 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.

Pursuant to this Study Plan, the Trustees plan to assess the following potential injuries to these birds: reduced avian reproduction and overt external malformations. The Trustees plan 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.

The Trustees intend to investigate injury to birds in the Hudson River system. The Trustees 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. 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 (DOI) contained in Title 43 of the Code of Federal Regulations (CFR) Part 11, Natural Resource Damage Assessment (NRDA). In 2004, work to document availability of nests and measure various parameters of kingfisher and sandpiper reproduction will be performed and pilot egg injection studies will begin. This work will continue in the year 2005 and potentially beyond, if the results of the work conducted in 2004 indicate that injury exists and further study is warranted. If necessary results of the 2004 work will be used to make changes in avian injury study design. This work will also guide the Trustees in the determination of potential restoration requirements for avian resources subject to identification and quantification of injury to avian resources.

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 (*Tachycineta bicolor*) from the Hudson River are known to be contaminated with PCBs. 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). Although there was not a statistically significant relationship between PCB concentrations in Hudson River tree swallows and reproductive parameters, there were high levels of nest abandonment and supranormal clutches that may be a response to PCB contamination (McCarty and Secord 1999a). Nest quality in these tree swallows, largely a reflection of adult behavior, was correlated with PCB concentrations, with lower quality nests (typified by less mass and fewer feathers) constructed at sites with greater PCB contamination (McCarty and Secord 1999b). Additionally, abnormal plumage development was noted in females at the Hudson River sites (McCarty and Secord 2000).

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*)). These six species were selected because together they provide a balanced approach in that these species use different types of habitats common to the Hudson River, they consume different types of foods and they generally represent different ecological guilds. Further, all six of these avian species are reported to be relatively common breeders in the Hudson River floodplain (Andrle and Carroll 1988) and use wetlands for some portion of their life cycle (DeGraaf and Yamasaki 2001). Finally, many of the prey species consumed by these six avian species include those for which PCB accumulation has been documented in other areas, and for which PCB accumulation in prey items from the Hudson River is likely to exist.

The 2002 avian egg exposure preliminary investigation also entailed collection of eggs 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 (Figure 1).

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, 2003a). Of the eleven species tested, spotted sandpiper eggs exhibited the highest individual egg concentration of PCBs (56 ppm, as total homologues, fresh weight basis) 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).

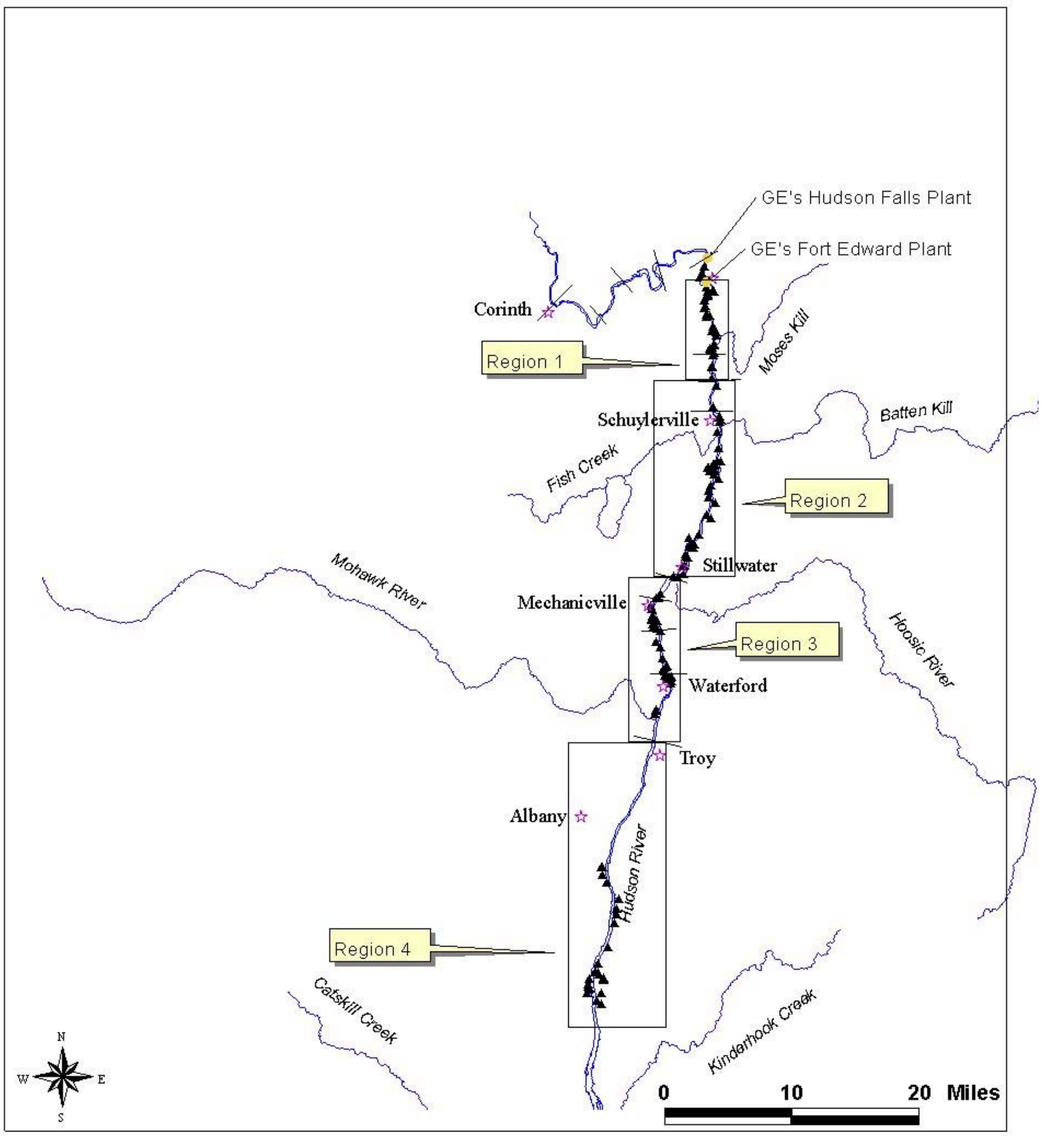

Figure 1. Location of avian egg collection sites depicted with triangles. Lines across the river represent dams or locks on the Hudson River. Stars represent select cities and towns.

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 2003a), 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 goals 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.

The work contained in this Final Study Plan is an avian injury investigation, and as such has a focus on identifying and determining potential injuries, and establishing causation from PCBs. This is in contrast to the Trustees' 2002 avian egg preliminary investigation, the purpose of which was to evaluate regional avian contamination with PCBs. Determining injury and establishing causation requires additional work and a scope of analytical work not necessarily warranted in a preliminary investigation.

Pursuant to the Hudson River NRDA Plan, the Trustees developed a Draft Study Plan for this avian injury determination effort, and engaged in peer and public review of that Draft Study Plan. The Draft Study Plan described the activities that constituted the Trustees' 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 asked the public and the party or parties responsible for the contamination to review the Draft Study Plan and provide feedback on the proposed approach. These Trustees sought public input to help them in planning and conducting an assessment that is scientifically valid, cost effective, and that incorporates a broad array of perspectives.

Accordingly, on March 15, 2004, a Draft Study Plan was released by the Trustees to the public for review and comment. A Notice of Availability of the Draft Study Plan was announced in the Federal Register on March 30, 2004. Availability of the Draft Study Plan was also announced by the Trustees on the Hudson River NRDA web sites maintained by the New York State Department of Environmental Conservation, the U.S. Fish and Wildlife Service (FWS), and the National Oceanic and Atmospheric Administration, and through a listserv maintained by the Trustees. A 45-day comment period was provided to the public, closing on April 29, 2004.

The Study Plan was also peer reviewed using an appropriate peer review mechanism by qualified specialists with necessary technical expertise. This level of peer review was sufficiently rigorous in light of the complexity of the study to ensure that the methods and study design were adequate to meet study objectives. Peer review of the work plan for the avian investigation took place in early 2004, and has been completed. Based on the peer review, modifications have been made to the Study Plan. The results and specific recommendations of the peer review will not be made available to the public because they are privileged pre-litigation materials.

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 commenter, 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 Final Study Plan for this effort was approved by the Hudson River Natural Resource Trustees on May 13, 2004, subsequent to approval by the Trustees' Quality Assurance Coordinator on April 8, 2004. The May 10, 2004 version of the Final Study Plan approved by the Trustees has been modified to produce this Final Study Plan, Public Release Version, dated June 15, 2004. The modification entailed replacing the section of the Trustees' May 10, 2004 Study Plan that described the USGS study of kingfisher, sandpiper and swallow with reference to the USGS Study Plan itself, and incorporation of that USGS Study Plan, including the signed approval page and the Technical Operating Procedure, into the Trustees' Final Study Plan as Appendix A.

Pursuant to this Study Plan, the Trustees plan to assess the following potential injuries to Hudson River belted kingfishers, spotted sandpipers and tree swallows: reduced avian reproduction and overt external malformations. The Trustees plan 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 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' Responsiveness Summary for the Hudson River NRDA Plan (Hudson River Natural Resource Trustees 2003b) provides additional details regarding the peer review process envisioned by the Trustees for such reports.

Other avian species not addressed in this Study Plan may be examined more closely at other stages of the NRDA and may be the subject of other Study Plans. Such work may focus on other migratory bird species, including endangered, threatened, and special concern bird species. The geographic scope of such studies may differ from that used in the work which is the subject of this Study Plan.

3.0 PURPOSE

The Trustees intend to investigate injury to birds in the Hudson River system. The Trustees 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. 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 (DOI) contained in Title 43 of the Code of Federal Regulations (CFR) Part 11, Natural Resource Damage Assessment (NRDA). In 2004, work to document availability of nests and measure various parameters of kingfisher, sandpiper, and swallow reproduction will be performed and pilot egg injection studies will begin. This work will continue in the year 2005 and potentially beyond, if the results of the work conducted in 2004 indicate that injury exists and further study is warranted. If necessary results of the 2004 work will be used to make changes in avian injury study design. This work will also guide the Trustees in the determination of potential restoration requirements for avian resources subject to identification and quantification of injury to avian resources.

4.0 METHODS

4.1 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.

The work has two objectives:

- determine if reproductive success of spotted sandpipers, belted kingfishers, and tree swallows nesting on the Hudson River is negatively affected by PCB exposure; and,
- determine organic contaminant accumulation rates in belted kingfisher chicks on the Hudson River.

Pursuant to the DOI NRDA regulations at Title 43 CFR Section 11.64(a)(2), in developing these objectives, consideration has been given to the availability of information from response actions relating to the hazardous substance release, the resource exposed, the characteristics of the hazardous substance, the potential physical, chemical, or biological reactions initiated by the hazardous substance release, potential injury, the pathway of exposure, and the potential for injury resulting from that pathway.

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

The USGS investigation entails the following components: assessment of reproductive success of belted kingfisher, spotted sandpiper and tree swallow, assessment of the incidence of gross deformities in embryos or hatchlings of these birds, and determination of organic contamination accumulation rates in belted kingfisher chicks.

The USGS Study Plan for this investigation, and the Technical Operating Procedure for that USGS Study Plan, describe this work, and are incorporated into this Final Study Plan as Appendix A.

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 affected by PCB exposure. The Trustees will also assess the incidence of gross deformities in embryos or hatchlings and, assess organic contamination accumulation rates in belted kingfisher chicks. The work on belted kingfisher chicks also supports a determination of pathway.

4.1.1 Hypotheses and Statistical Tests

The Principal Investor (PI) plans to conduct the following comparisons. Null (HO) and alternative (HA) hypotheses are presented below.

- Compare contaminant levels in eggs between "successful" and "failed" nests for each species in the contaminated portion of the river and between sites (i.e., the contaminated site and one or two reference areas) (see Blus *et al.* 1974).

  o General Hypotheses

  - H_O: Mean $\text{Level}_{\text{successful}}$ = Mean $\text{Level}_{\text{failed}}$ (and between sites)

  - H_A: Mean $\text{Level}_{\text{successful}}$ ≠ Mean $\text{Level}_{\text{failed}}$ (and between sites)

  o Statistical tests

  - A two-way analysis of variance (ANOVA) will be used to compare total PCB (tPCB) levels between nest categories (i.e., "successful" or "failed") and site (i.e., contaminated portions of the river and one or more reference areas) for each study species. The data may be log-transformed prior to running the analysis (Custer *et al.* 2003a; Hudson River Natural Resource Trustees 2003a). If there is a significant interaction between nest categories and site, one-way ANOVA will be used to determine which combinations are significantly different (Custer *et al.* 2003a). The analysis will be followed by a Bonferroni mean separation test to determine which means were significantly different.

- Compare reproductive success in relation to contaminant levels.

  o General Hypotheses

  - H_O: Mean Hatching $\text{Success}_{\text{contaminated}}$ is not proportionally related to tPCB levels

  - H_A: Mean Hatching $\text{Success}_{\text{contaminated}}$ is proportionally related to tPCB levels

  o Statistical tests

  - Hatching success in relation to contaminant levels will be evaluated using logistic regressions of hatching success (dependent variable) and contaminant levels (independent variable). The dependent variable for these analyses will be a binomial (i.e., "successful" vs. "failed" nests) and the independent variable will be the log-transformed primary contaminant data (e.g., tPCB).

- Compare daily egg survival (p) between sites.
  - o General Hypotheses
    - H_O: $p_{contaminated} = p_{reference}$
    - H_A: $p_{contaminated} \neq p_{reference}$
  - o Statistical tests
    - Methods developed for the Mayfield estimator by Hensler and Nichols (1981) will be used, as well as methods for comparing survival rates described by Sauer and Williams (1989).

- Compare contaminant accumulation rates (r) in belted kingfisher chicks between sites.
  - o General Hypotheses
    - H_O: $r_{contaminated} = r_{reference1} = r_{reference2}$
    - H_A: $r_{contaminated} \neq r_{reference\ 1} \neq r_{reference2}$
  - o Statistical tests
    - Accumulation rates will be compared using one-way ANOVAs.

These hypotheses and statistical tests may be revised, or not performed, by the PI based on data collection. Further, the PI may test other hypotheses and conduct additional statistical tests not noted above.

4.1.2 *Quality Assurance Plan*

Section F of the USGS Study Plan (Appendix A) contains the Quality Assurance/Quality Control Plan (QAP) for the USGS investigation. This QAP has been prepared pursuant to the DOI NRDA regulations which specify, at Title 43 CFR Section 11.31(c)(2), that, "If the authorized official plans to use type B procedures, the Assessment Plan must include a Quality Assurance Plan that satisfies the requirements listed in the NCP and applicable EPA [U.S. Environmental Protection Agency] guidance for quality control and quality assurance plans." As noted in the Trustees' Responsiveness Summary for the NRDA Plan (Hudson River Natural Resource Trustees, 2003b), for each data collection effort that is part of the Hudson River NRDA and is identified in the NRDA Plan, the Trustees will develop a project-specific QA Plan which may be an independent document or may be incorporated into the project Study Plan. Such a QA Plan, in combination with the information on QA management described in the NRDA Plan (Hudson River Natural Resource Trustees, 2002a), will ensure that the requirements listed in the National Contingency Plan and applicable EPA guidance for quality control and quality assurance plans are met.

The Data Generation and Acquisition section of the QAP (section F.2 of the USGS Study Plan contained in Appendix A) addresses the quality of field-collected data. The assessment of quality of analytical data is addressed in the Hudson River NRDA Analytical Quality Assurance (QA) Plan (Hudson River Natural Resource Trustees, 2002b), which will be updated to include measurement quality objectives for any additional analytes not in the current plan. Laboratories will provide fully documented data packages which will enable data validation to be performed based on the criteria provided in the Analytical QA Plan for the Hudson River NRDA, applicable laboratory Standard Operating Procedures, and the U.S. Environmental Protection Agency (1999). The Trustees will release the updated Analytical QA Plan when it is finalized.

4.2 Avian Egg Injection Pilot Study

Egg injection assesses the effects of contaminants on a developing avian embryo. Avian egg injection is a well-established technique to assess the effects of contaminants on a developing avian embryo. Conducting such work with eggs of belted kingfisher and spotted sandpiper, for example, would elucidate the effect of PCBs on developing embryos of these species. Results of injecting contaminants, such as PCBs, into avian eggs include embryomortality and malformation. Death, such as embryomortality, and physical deformation, such as external malformation, skeletal deformities, and organ and soft tissue malformation, are injuries pursuant to the DOI NRDA regulations, and would be relevant to determining injury and establishing causation.

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.

The avian egg injection pilot study itself is a preliminary investigation; no Study Plan for such will be released for public review and comment, in accordance with the Hudson River NRDA Plan. The Hudson River NRDA Plan specifies different requirements for peer and public review of work plans for injury determination studies (such as the work being done by USGS pursuant to this Study Plan) and preliminary investigations (such as the egg injection pilot study).

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. Should the Trustees determine, based on the results of the pilot study initiated in 2004, that it is appropriate to conduct a full-scale egg injection study, a Study Plan would be prepared, and such a Plan would be peer reviewed and provided to the public for review and comment, in accordance with the Hudson River NRDA Plan.

5.0 LITERATURE CITED

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Appendix A

USGS Study Plan, Including Hudson River Avian Study Technical Operating Procedure (Standard Operating Procedure No. TS 404.0)

Exposure and effects of PCB contamination on Spotted Sandpipers, Belted Kingfishers, and Tree Swallows on the Hudson River

USGS Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd., La Crosse, WI 54603

Sponsor

Natural Resource Damage Assessment
HUDSON RIVER NATURAL RESOURCE TRUSTEES

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

UMESC Study number	TS-04-2282-9RZ38-01
Basis Code:	~~2282~~ 8335 -9RZ38 KG 6/10/04
Proposed Start Date:	April 2004
Proposed Termination Date:	January 2007

5/26/04
Patricia Heglund, Ph.D.
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Christine Custer, Ph.D.
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6-7-04
Date

Brian Gray, Ph.D.
Statistician

6/7/04
Date

Quality Assurance Coordinator

June 11, 2004
Date

Leslie Holland-Bartels, Ph.D.
Center Director

06/04/04
Date

Approved by:

Sponsor
Hudson River Natural Resource Trustees

6/15/04
Date

Study Director
Thomas Custer, Ph.D.

05/29/2004
Date

A. Project

Title: Exposure and effects of PCB contamination on Spotted Sandpipers, Belted Kingfishers, and Tree Swallows on the Hudson River

PI: Thomas W. Custer, Wildlife Biologist, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603.

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Facility Name and Address: USGS, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603

BASIS Number: TBD

Task Number: TBD

B. Project Summary:

Scientists from the U.S. Geological Survey's Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, plan to conduct a study in the Spring and Summer 2004 of spotted sandpipers (*Actitis macularia*), belted kingfishers (*Ceryle alcyon*), and tree swallows (*Tachycineta bicolor*) on polychlorinated biphenyl (PCB)-contaminated portions of the Hudson River, New York in Spring-Summer 2004. The primary objective is to determine whether reproductive success of spotted sandpipers, belted kingfishers, and tree swallows is negatively affected by PCB exposure on the Hudson River. For spotted sandpipers and belted kingfishers, the work will be conducted on the Hudson River starting at Bakers Falls and extending south up to 43 river miles to Lower Schodack Island. For tree swallow, nest boxes will be positioned near areas on the Hudson River where contamination with PCBs is suspected. For all species, areas farther north on the river and nearby rivers and wetlands may be included as reference locations. A sample egg or eggs will be randomly collected from each nest and success of the remaining eggs will be monitored to assess reproductive success. Organic contaminant concentrations in the sample eggs will be compared among nests differing in reproductive success. A second objective is to document local exposure to PCBs of birds nesting on the Hudson River. Kingfisher chicks will be collected and analyzed for organic contaminants including total PCBs. Contaminant accumulation rates will be determined and compared among sites.

C. Problem

The General Electric Company is believed to have discharged between 0.2 and 1.3 million pounds of PCBs into the Hudson River between the 1940s and 1977 (Baker *et al.* 2001). These PCBs have been detected in the sediment, water, and biota of the Hudson River at levels of potential ecological concern (TAMS Consultants, Inc. and Menzie-Cura & Associates, Inc. 2000). A recent study documented elevated PCB levels in Hudson River floodplain soils (S E A Consultants, Inc. 2002). As a result of PCB contamination from this release, the State of New York acting through the New York State Department of Environmental Conservation, the Department of the Interior acting through the U.S. Fish and Wildlife Service and the National Park Service, and the Department of Commerce acting through the National Oceanic and Atmospheric Administration (Hudson River Natural Resource Trustees [Trustees]) are conducting a natural resource damage assessment of the Hudson River.

A number of studies have documented that bird species can absorb PCBs from prey items and feed (Custer *et al.* 1997, 1999, and 2003a, Drouillard and Norstrom 2001, Froese *et al.* 1998, Larson *et al.* 1996, and Secord *et al.* 1999). Numerous studies have further shown that a species' position within the food chain affects the amount of PCBs that it will bioaccumulate (Focardi *et al.* 1988, Senthilkhumar *et al.* 2002, Zimmerman *et al.* 1997). In particular, avian species that primarily feed on fish or other birds tend to accumulate PCBs to a greater extent than do species that feed at lower trophic levels (Forcardi *et al.* 1988, Prestt *et al.* 1970, Senthiklumar *et al.* 2002).

In either adults or eggs, PCBs can reach concentrations that may cause toxic effects (Fernie *et al.* 2001, Hoffman *et al.* 1998, Stratus Consulting, Inc. 1999). Generally speaking, these toxic effects can include mortality, malformations, decreased body weight, and/or reproductive impairment. The nature and severity of the effect depends on the species, dosage, PCB congener(s) composition, and the birds' physiology (Barron *et al.* 1995, Eisler 2000, Eisler and Belisle 1996, Fernie *et al.* 2001, Environment Canada 1998, and Hoffman *et al.* 1996a, 1996b, 1998). Effects of PCBs on adult birds' reproductive behavior include increased amounts of time spent in the courtship phase, reduced pair bond formation, delayed nest building, building nests of poor quality, delayed egg laying, burying eggs in nesting material, decreased nest attentiveness, inconsistent incubation causing fluctuations in egg temperatures, and increased nest abandonment (Arena *et al.* 1999, Fernie *et al.* 2001, McCarty and Secord 1999a and 1999b, Peakall and Peakall 1973, Tori and Peterle 1983).

Mortality, deformity, and other toxicological effects to embryos and nestlings are correlated with PCB contamination in eggs; again, effects vary according to species sensitivity, levels of contamination, and composition of PCB mixtures (Brunstorm and Reutergardh 1986, Brunstorm 1989, Bush *et al.* 1974, Hill *et al.* 1975). Known effects include decreased hatching success, delayed hatching, increased embryonic deformity rates (i.e., 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).

Spotted sandpipers, belted kingfishers, and tree swallows consume different types of foods and generally represent different ecological guilds. Spotted sandpipers are ground foraging insectivores, often in 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). Tree swallows are aerial feeding insectivores feeding mainly on emergent aquatic insects (Blancher and McNicol 1991)

Spotted sandpipers, belted kingfishers, and tree swallows are suitable for study because they are reported to be relatively common breeders in the Hudson River floodplain (Andrle and Carroll 1988). As stated earlier, PCB contamination of Hudson River floodplain soils was recently documented (S E A Consultants, Inc. 2001).

Few field studies of the effects of PCBs on Hudson River bird populations exist. One recent set of studies examined tree swallows in the Hudson River basin. Tree swallows nesting along the upper Hudson River had PCB concentrations up to 114 μg/g for 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 μg/g, while concentrations in nestlings ranged from 3.7 to 62.2 μg/g (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). Tree swallows have been able to successfully reproduce with PCB concentrations that cause 100% embryo mortality in more sensitive species (McCarty and Secord 1999a). McCarty and Secord (1999b) also described abnormal nesting behavior of tree swallows and inferred that chemical contamination can possibly interfere with behavior.

PCB concentrations were elevated in spotted sandpiper and belted kingfisher eggs collected in 2002 from Bakers Falls to Lower Schodack Island on the Hudson River (Figure 1). 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 μg/g PCBs (as total homologues, fresh weight basis). Of the eleven species tested, spotted sandpiper eggs exhibited the highest individual egg concentration of PCBs (56 μg/g) as well as the highest average PCB concentration (15 μg/g). Of the eleven species tested, belted kingfisher eggs exhibited the second highest individual egg concentration of PCBs (43 μg/g).

These elevated PCB concentrations were similar to levels associated with reduced reproductive success in other species. For tree swallows on the Housatonic River, MA, hatching success was depressed beginning at 20 and 50 μg/g PCBs (wet weight) (Custer *et al.* 2003a). The sensitivity of spotted sandpipers and belted kingfishers to PCB contamination relative to that of tree swallows is unknown.

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.

The number of spotted sandpiper and belted kingfisher nests available for a study of the effects of PCBs on reproduction from Bakers Falls to Lower Schodack Island of the Hudson River is unknown. In 2002, eggs were collected from this stretch of the river from 13 spotted sandpiper and 9 belted kingfisher nests. The contractor, who collected the 2002 eggs, suggested that with additional effort perhaps 20 belted kingfisher nests and 25 spotted sandpiper nests could be found in Regions 1 to 4 (Figure 1).

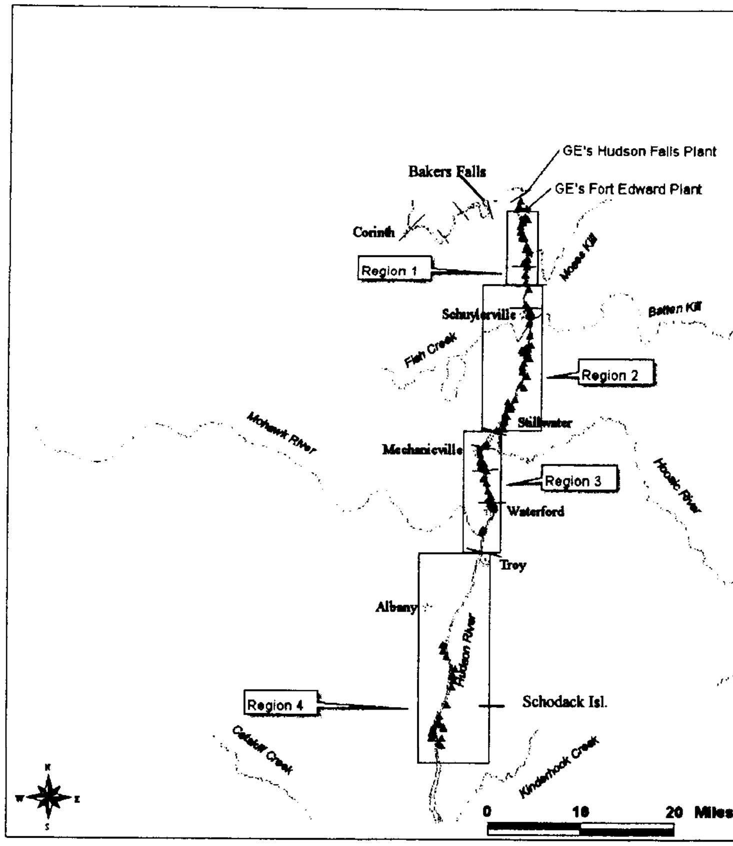

Figure 1. Location of avian egg collection sites depicted with triangles. Lines across the river represent dams or locks on the Hudson River. Stars represent select cities and towns.

D. Objectives:

1. Determine if reproductive success of spotted sandpipers, belted kingfishers, and tree swallows nesting on the Hudson River is negatively affected by PCB exposure.

2. Determine organic contaminant accumulation rates in belted kingfisher chicks on the Hudson River.

E. Methods:

1. Determine if reproductive success of spotted sandpipers, belted kingfishers, and tree swallows nesting on the Hudson River is negatively affected by PCB exposure.

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 or eggs (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, (Gilbertson *et al.* 1991, Fernie *et al.* 2003, Hoffman *et al.* 1998, Kuiken *et al.* 1999, Larson *et al.* 1996, Ludwig *et al.* 1993, Powell *et al.* 1996, Summer *et al.* 1996) where possible.

Overview

For spotted sandpipers and belted kingfishers nest surveys will focus on the Hudson River from Bakers Falls to Lower Schodack Island (Figure 1), on the reach upstream of Glens Falls as a reference location, and possibly on a neighboring reference river (Connecticut River or others).

Avian investigations conducted previously by the Trustees have defined the following Regions:

Region	Region Name	Region Description	Starting Latitude	Ending Latitude
1	Thompson Pool And Vicinity	Starts at Bakers Falls and ends at Champlain Canal	73.5855	73.5784
2	Stillwater Pool and Vicinity	Starts at Champlain Canal and ends at Lock No. 4	73.5784	73.6521
3	North of Troy and Vicinity	Starts at Lock No. 4 and ends at Peebles Island State Park	73.6521	73.6790
4	South of Albany and Vicinity	Starts at Albany City South Boundary and ends at Lower Schodack Island	73.7650	73.7910
5	Upstream Reference Region	Upstream of Baker's Falls	Any area north of 73.5855	Not applicable
R	Other Reference Region	All not immediate Hudson River locations	Not applicable	Not applicable

This work will be carried out by using geo-referenced data for previous nest locations, combined with field notes and information from interviews and/or cooperation with previous contractors. Further field work will be carried out by searching for nests using experienced field crews in boats, vehicles and by foot.

Maps showing the location of areas to survey for the two study species will be compared to land ownership maps obtained from the New York State GIS Cooperative (NYSGIS) and local assessors' offices. Accessible public lands with suitable habitat will be surveyed for study locations. Private landowners will also be approached for permission to search for nests where suitable nesting habitat occurs on private lands. Methods of contact will include a combination of phone calls, letters, and personal contact.

For tree swallows, 150 or more swallow boxes, 30 or more at each of 3 or more sites, will be attached to posts, trees, or other suitable structures in suitable habitat. Predator guards will be used as needed. Boxes will be placed approximately 30 m apart, but this can vary depending on the structure of the habitat. Additional boxes may be added in subsequent years of the study. Two or more sites will be below Glens Falls on the Hudson River and one site, the reference, will be on a nearby river. Location of boxes and specific study sites will be determined during the first visit to the area, but may be modified in subsequent years of the study based on the professional judgment of the Principal Investigator.

Once nests are located they will be visited and clutch size, hatching success, and fledging success (kingfishers and swallows only) determined. The location of nests sites will be documented in Universal Transverse Mercator Grid values by Global Positioning System (GPS) units. Egg weights for collected eggs and incidence of gross deformities (Gilbertson *et al.* 1991, Fernie *et al.* 2003, Hoffman *et al.* 1998, Kuiken *et al.* 1999, Larson *et al.* 1996, Ludwig *et al.* 1993, Powell *et al.* 1996, Summer *et al.* 1996) in embryos or hatchlings will be documented. 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 within each year separately and for both years combined.

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). Toxic equivalent values will be calculated using World Health Organization (WHO)-consensus toxic equivalency factors for PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) (Van den Berg 1998).

Egg collection will be conducted pursuant to the Hudson River Avian Study Technical Operating Procedure, prepared by the U.S. Geological Survey's Upper Midwest Environmental Sciences Center (Standard Operating Procedure (SOP) No. TS 404.0). Egg samples will be maintained under chain of custody upon collection, and through processing, storage and shipment to the analytical laboratory, according to Quality

Assurance/Quality Control provisions of this work plan (section F). Egg contents will be removed in a field laboratory, stored frozen (-20 degrees C), then shipped to the Trustees' analytical laboratory. Egg content 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 estimated number of eggs collected for chemical analysis for kingfishers (n=60, Table 1) is based on 20 from the study area, 20 from reference areas, and 20 found abandoned or unhatched in successful nests. The estimated number of eggs for sandpipers (n= 60, Table 1) is based on 25 from the study area, 25 from reference areas, and 10 found abandoned or unhatched in successful nests. The estimate number of diet samples (n=10, Table 1) is based on half of the chicks having sufficient food mass for chemical analysis.

Table 1. Estimated number of samples to be collected for chemical analysis in 2004.

Species	Matrix	Analysis	FY04 samples
Spotted Sandpiper	Egg	Organochlorines, PCB congeners, dioxins, and furans	60
Belted Kingfisher	Egg	" " "	60
	Chick Carcass	" " "	20
	Diet	" " "	10

The number of nests necessary to adequately test whether hatching success is related to PCB exposure will be estimated using a power analysis (Cohen 1988). The power analysis will be based on PCB concentrations in spotted sandpiper and belted kingfisher eggs collected from the Hudson River in 2002 and the relationship between PCB concentrations in sample eggs and hatching success identified for tree swallows on the Housatonic River (Custer *et al.* 2003a).

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.

Spotted Sandpiper

Visual observations and listening for sandpiper alarm calls will be used to locate potential breeders. Observing these individuals will help locate nests although extended observations may be needed to follow the birds to areas where nests may be present. The presence of more than one spotted sandpiper in an area may also be indicative of breeding or nesting activity in that area. Nests are usually next to and concealed by a dense tuft of grasses or sedges. Heat sensing devices may be used to enhance finding sandpiper nests (Galligan *et al.* 2003), particularly in the early morning when temperatures are cool and nests and eggs are substantially warmer.

A sample egg will be randomly collected from each nest as soon as the clutch is complete. Females generally lay 4 eggs; the average interval between laying and successive eggs is 27 hours (Oring *et al.* 1997). When the number of eggs in the clutch remains the same for more than 48 hours it will be considered complete. Because spotted sandpiper chicks may leave the nest within hours of hatching (Oring et al 1997), several methods will be attempted to be determine whether eggs hatched or were depredated. Nests will then be monitored closely in the final days of incubation. Time-lapse photography may also be used to determine hatching success; if time-lapse photography will be used, an amendment to this plan will be prepared addressing the specifics of its use. Nests may be fenced in to contain the chicks until they can be accounted for; if fencing is used, an amendment to this plan will be prepared addressing the specifics of its use and routed through the ACUC committee.

Belted Kingfisher

Once kingfisher pairs are located they will be observed at perches and their movements tracked to locate nest sites. Nests will be monitored so that visits can be timed to prevent nest disturbances as much as possible. A sample egg will be randomly collected late in incubation. Belted kingfisher clutches generally have 5 to 8 eggs, most commonly 6 or 7 eggs (Hamas 1994). Because kingfishers may abandon their nests if disturbed early in incubation (Hamas 1975), nest monitoring will be based on the protocol developed by Kelly and Van Horne (1997). Kelly and Van Horne (1997) visited the nest site every fourth day and recorded behaviors of the adults. They noted that the presence of the female perched outside the burrow for long periods of time was an indicator of egg-laying. Combining this behavioral information with the length of the incubation period (22 days after the final egg is laid [Hamas 1994]), we will predict the stage of incubation and time the first nest visit for the end of the incubation period. At that time, the nest will be viewed with a burrow scope (Henning and Brooks 2002) or access to the nest obtained by digging an accessway through which an egg can be retrieved and the nest monitored (Albano 2000). If a hole is dug, it will be covered to protect the nest against weather and predators. To avoid potential disturbance of archaeologically significant areas, no digging will be conducted at nests located in Saratoga National Historic Park.

In situations where it is unsafe to access a burrow or for burrows that are too deep or otherwise unsuitable for retrieving an egg, the survey team will not attempt to retrieve an egg. Such nests will not be monitored.

Tree Swallow

Tree swallow nest boxes would be set along the Hudson River in the study area, and birds that use those boxes monitored for hatching success, fledging success and nestling gross deformities, with collection of eggs following the sample egg protocol of Blus *et al.* (1974). Each nest box will be visited approximately once per week until egg laying begins. After that time, nests will be visited up to 2 - 3 times per week, or more often as needed to collect eggs or just hatched young samples. After the eggs have hatched, boxes will be visited at least once per week until the young reach 12 days of age. Whether eggs or young are present in the nest box and the number of eggs and young present will be recorded on data sheets (Attachment A).). A sample of 2 eggs and/or just-hatched eggs (hereafter termed pippers) will be collected from each nest. Analysis of eggs (sample eggs or failed eggs) would take place only if determined warranted by the Trustees, such as if substantial numbers of failed clutches or gross deformities are observed.

2. Determine organic contaminant accumulation rates in belted kingfisher chicks on the Hudson River.

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. Kingfisher nestlings will be collected either by hand through a tunnel at the back of the nest or with a hook used in conjunction with the burrow scope.

Nestlings will be collected, processed, and euthanized pursuant to the Hudson River Avian Study Technical Operating Procedure (SOP No. TS 404.0). Chain of custody for the nestlings will be maintained upon collection through euthanization, processing, storage and shipment to the analytical laboratory, according to Quality Assurance/Quality Control provisions of this work plan (section F). Samples from nestlings will be frozen, and shipped to the Trustees analytical laboratory for analysis. Nestling 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. 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).

F. Quality Assurance/Quality Control Plan

F. 1. Project Management

This study is being conducted in accordance with the Quality Assurance Management Plan for the Trustees' Hudson River NRDA (Hudson River Natural Resources Trustees, 2002). The study team is organized based on tasks and levels of responsibility to ensure good communication between all personnel. The Assessment Managers (Kathryn Jahn, USFWS, Tom Brosnan, NOAA, and Larry Gumaer, NYSDEC) have overall project oversight responsibility and provide direction to the Quality Assurance Coordinator (Ann Bailey, EcoChem Inc.). The Principal Investigator (PI, Dr. Thomas W. Custer) and Co-Investigator (CI, Dr. Christine M. Custer) are responsible for the project's design and implementation. They provide guidance and technical expertise as needed to the field teams that consist of biologists, technicians, and cooperators on the project.

The PI or designee provide instructions to field teams on all aspects of the project, including quality assurance management. Field team members report to the PI or designee who will work with the Assessment Managers and Quality Assurance Coordinator to ensure that the study is consistent with the overall QA objectives of the NRDA.

The Study Plan and Standard Operating Procedures for this study were developed to provide detailed and explicit instructions for the field crews to follow when collecting study data. The plan has been reviewed, commented on, and approved by key parties to the study before the beginning of sample collection. Reliance on a detailed, explicit, and fully reviewed study plan ensures that:

- Study objectives, methods, procedures, and details are reviewed thoroughly before sampling.
- Data will be collected in a systematic and consistent way throughout the study.
- Every member of the study team adheres to the requirements of the plan. Each field team member is required to sign a statement (Attachment B) that they have read the Study Plan and associated SOPs (TS 404.0) and understand them.

Events can arise during field data collections that require changes to the procedures being used. In these circumstances, deviations from the plan will be conducted only after consultation between the PI or designee. Deviations from the work plan will be carefully documented, as will a detailed explanation as to why the deviations were necessary.

F. 2. Data Generation and Acquisition

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, and be consistent with sound scientific methodology appropriate to the data quality objectives.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification (e.g., distinguishing between a similar species) will not be possible for specimens that are not collected, however, agreement between field team members regarding species identification must be obtained for verification. This will occur in the field on a daily basis as data are collected. The frequency and type of field checks are listed in Table 2.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to the field portion of this study, accuracy means that the target animals and their nests are correctly identified. Accuracy for analytical data collected as part of egg analysis will be defined in the Hudson River NRDA Analytical QA/QC Plan.

Completeness is defined as the percentage of the planned samples actually collected and processed. Although sample sizes cannot be predetermined, observations must be conducted throughout the season when the breeding birds are present in the study area and in each habitat that these species could use where access is granted. The full distribution of study efforts within those parameters is a measure of the completeness of this study.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Obtaining representative data for this study will be ensured through the establishment of a thorough literature review to identify life history characteristics, breeding habitat, and nest site descriptions, and by completing field investigations in a manner to determine if those species are present.

Comparability is defined as the measure of confidence with which results from this study may be compared to another similar data set. Because of the nature of the study, there cannot be a duplication of effort in the same area at the same time. Comparability will be attained through use of standardized, peer-reviewed field techniques that are commonly used in these types of studies in different parts of North America.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability to locate and identify the three study species and their nests. This process is a stepwise approach that requires ornithological expertise. First, potentially suitable habitat must be located through the use of general habitat reconnaissance and specific habitat assessment. Once suitable breeding habitat is located, breeding bird surveys can begin. Surveys involve using visual searches and silent listening to locate the species for which there is potentially suitable habitat. In many cases, a bird will be identified through silent listening by a territorial, breeding, or alarm call or song. Furthermore, the surveyor must be able to follow the individual bird and locate the nest site and nest. Therefore, species identification includes knowledge of songs and calls, visual identification, territorial behavior, habitat use, nest site selection, and nest and egg identification.

Table 2. QAP Type of Field Checks and Frequency.

Type of Field Activity	Measurement	Minimum Frequency of Check by PI or designee	Acceptance Criteria
Study species identification by sight	Each study species can be identified by sight and using a field guide for confirmation.	At the beginning of study photographs, slides, and /or video images of the three study species will be used to check identification.	One hundred percent accuracy on identification.
Study species nest and egg identification	Each study species nests and eggs can be identified by sight and using a field guide for confirmation.	At the beginning of study photographs, slides, and/or video images of the three study species will be used to check identification.	One hundred percent accuracy on identification.
GPS data collection (including error) and data downloading	Field personnel can operate GPS equipment and transfer data to computers.	Once before beginning the study, and then monthly as data is downloaded and verified.	Electronic records are kept up to date and a paper copy is created and filed with the study records.
Completion of data forms	Data forms are filled out each day correctly and completely.	Weekly.	Data forms are complete, legible, and accurate.
Sample collection	Eggs and nestlings are properly labeled when collected and then transferred to lab for preparation.	Each day an egg or nestling is collected.	Each egg or nestling is correctly assigned a sample ID number.
Sample preparation	Eggs or nestlings are processed according to this SOP.	Each day eggs or nestlings are processed or every 20 samples.	Egg and nestling samples are prepared for shipment to analytical laboratories or archive freezer.

F. 3. Study Documentation

All study activities will be documented in field notebooks, data forms, or personal digital assistants (PDAs) as appropriate. Electronic files will be downloaded and hardcopies printed off. All hardcopies will be placed into 3-ring or other binders. To the extent possible, information will be recorded on pre-formatted data sheets. The use of pre-formatted data sheets is a QA/QC measure designed to:

- ensure that all necessary and relevant information is recorded for each sample and each sampling activity,
- serve as a checklist for the field crews to help ensure completeness of the data collection effort,

- assist the field crews by making data recording more efficient, and
- minimize the problem of illegible field notebook entries.

Each field crew will have a single field data recorder responsible for recording information in field notebooks or on data forms. Assigning this responsibility to a single person will help ensure that documentation is complete and consistent throughout the sampling event. The field data recorder is also responsible for the care, custody, and disposition of the field notebook and data forms and for downloading electronic files and providing hardcopies.

Field notebook and data sheet entries will be made in ink. Corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each completed data sheet will be reviewed, corrected (if necessary), and initialed by the field data recorder. Following completion of the study, field notebooks, data sheets, and electronic files originals will be stored at the Upper Midwest Environmental Sciences Center, La Crosse, WI.

F. 4. Personnel Experience and Training

Field crews will receive explicit instructions in the execution of the Study Plan and SOPs. The field crews will be instructed in the field before beginning any sampling, and the instructions will be repeated or refreshed during the sampling period as necessary (Table 2). Field-crew members will be trained to identify each study species by sight, their habitat, nests, and eggs.

F. 5. Assessment and Oversight

The QC management plan specifies that studies that generate data will be audited to ensure that the project-specific plans are being properly implemented. Several mechanisms for internal audits of the data generation process will be used for the breeding bird egg exposure study. These mechanisms include:

- A project management structure that defines clear lines of responsibility and ensures communication between field crews and the PI or designee. Clear responsibilities and communication can serve as a means of providing internal audits of the sample collection process as it proceeds.
- A requirement that field notebooks and data forms be reviewed periodically by the PI or designee.
- The use of pre-formatted data sheets that serve as a checklist for sampling procedures, thereby helping to ensure that sampling is complete.
- The sampling will not begin until approval is received from the Quality Assurance Coordinator or their delegate. The Quality Assurance Coordinator or her designee will conduct a field audit of procedures and documentation of the study.

F. 6. Data Validation and Usability

This study employs standard, repeatable methods available in the scientific literature for collecting data. The work plan for this survey has been extensively reviewed for the adequacy of the sampling design and methods. The original field notebooks will be maintained by USGS, Upper Midwest Environmental Sciences Center and archived for a minimum of eight years. Disposal of the notebooks will be coordinated with USFWS after this timeframe unless a longer archive period is requested. Final reports can then be reviewed against the sampling records to ensure that the data presented in the reports represent complete and accurate information. Validation of analytical data will be based on the criteria contained in the Trustees' Analytical QA Plan, which will be updated to include measurement quality objective for any additional analytes not in the current plan, as well as applicable laboratory Standard Operating Procedures, and the U.S. Environmental Protection Agency (1999) guidelines.

The PI or designee will validate that biologists and technicians are correctly identifying the proper species and completing data forms correctly by performing periodic checks during surveys.

Data analysis will be performed using SAS or other commercially available statistical software. Collected data will be summarized using measures of central tendency (e.g., means, medians) and variability (e.g., parametric or nonparametric confidence intervals).

F. 7. Chain of Custody Procedures

Strict Chain of Custody (COC) procedures will be used throughout the study. The purpose of COC is to assure the integrity of each sample and be able to clearly identify who was responsible for the sample at each step. The COC procedure will begin when an egg or nestling is collected from the nest. That collection is documented on field data forms (Form TS 4040a), which clearly identify the team member(s) responsible, as well as the date and time. The egg and nestling dissection forms (Forms TS 404.0b and 404.0c) will clearly identify to whom the sample was delivered for further processing, and will also include the date and time. The COC form (Attachment C) will be used to maintain records of sample transfer between personnel other than immediate team members.

The immediate team members are personally responsible for the care and custody of the samples that are in their possession. A sample is in custody of the immediate team member if any of the following occur:

- The sample is in the individual's physical possession;
- The sample is within view after being in possession;
- The sample is in a locked or sealed container that prevents tampering after being in possession; or,
- The sample is in a designated secure area.

When the samples are packed in coolers or other containers for shipment to the laboratory or storage facility, completed COC records (Attachment C) will accompany the samples. The COC form will contain the following information:

- Project name;
- Sample identification (unique for each sample);
- Sample matrix (e.g., egg contents, liver) which may be part of the sample ID;
- Name and signature of individual relinquishing custody;
- Name and signature of individual accepting custody;
- Sample shipping date and mode.

Other information such as date of sample collection, collection location, and jar sizes may be on the COC form or on accompanying documentation.

Each shipping container containing samples will be accompanied by an original COC record for the samples in that cooler. All sections of the COC form will be completed. All samples included in the sample catalog will be clearly listed. Indication of the number of coolers per shipment (e.g., 1 of 3) will be listed on the form if more than 1 container is shipped. Once the form is completely filled out, it will be placed securely inside the cooler (in a plastic sealable bag to keep it dry). Field personnel will maintain a copy if the COC to keep with the airbill. The cooler will be sealed with custody seals or the containers inside the cooler may be sealed with custody seals. Custody seals are used to detect unauthorized tampering with samples after sample collection until the time of use or analysis. Signed and dated gummed paper seals may be used for this purpose. The seals will be attached so that they must be broken to open the shipping container Each cooler will be sturdy and well sealed with strapping or other tape. All samples will be kept in locked locations or with custody seals at all times until shipped.

An air bill, Federal Express shipping label, etc. can be used to document the transfer of a sample from the field team to an intermediate storage location, the analytical laboratory, or archive freezer.

Coolers or other containers containing samples will be opened at the analytical laboratories or archiving facility only by a person authorized to receive the samples. The containers will first be inspected for integrity of the chain of custody seals or other signs of tampering. The receipt of each sample in the coolers or containers will be verified on the COC forms. The signed COC forms will be photocopied, and the photocopy will be mailed to the sending party. Samples will be stored in a secure area according to procedures documented for each analytical facility.

G. 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 will be reviewed and approved by the Animal Care and Use Committee, U.S Geological Survey, Upper Mississippi Science Center, La Crosse, WI, prior to beginning collection

H. Staff

Principal Investigator: Thomas W. Custer, USGS, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603. 608 781-6375 (voice); 608 783-6066 (fax); tcuster@usgs.gov

Coinvestigator: Christine M. Custer, USGS, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603. 608 781-6247 (voice); 608 783-6066 (fax); ccuster@usgs.gov

Coinvestigator: Brian Gray, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603. 608 781-6234 (voice); 608 783-6066 (fax); brgray@usgs.gov

Assistant: Craig Beckman USGS, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603. 608 781-6329 (voice); 608 783-6066 (fax); cbeckman@usgs.gov

Assistant: Steve Houdek, USGS, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603. 608 781-6305 (voice); 608 783-6066 (fax); shoudek@usgs.gov

Assistant: Paul Dummer, USGS, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd., La Crosse, WI 54603. 608 781-6241 (voice); 608 783-6066 (fax); pdummer@usgs.gov

Thomas Custer has primary responsibility for project design of avian sampling, coordination of avian field collections and sample processing, data interpretation (including statistical analyses) and scientific publications resulting from the avian investigation. Additionally, he will have lead responsibility on kingfisher and sandpiper portions of the study.

Christine Custer will assist with responsibility for project design of avian sampling, coordination of avian field collections and sample processing, data interpretation (including statistical analyses) and scientific publications resulting from the avian investigation. Additionally, she will have lead responsibility on tree swallow portion of the study.

Brian Gray will offer statistical assistance on all phases of the study.

Craig Beckman will serve as leader of the spotted sandpiper field survey team. He will coordinate and participate in all field activities including nest surveys, field collections and sample processing.

Steve Houdek will serve as leader of the belted kingfisher field survey team. He will coordinate and participate in all field activities including nest surveys, field collections and sample processing.

Paul Dummer will assist part-time as needed on both field survey teams. He will assist in avian field collections, sample processing, and statistical analyses.

I. Cooperators

U.S. Fish and Wildlife Service Personnel will provide logical assistance as needed. This may include providing information from the 2002 egg collection, habitat and land ownership maps, and use of boats and other supplies. Additional assistance may be provided by USFWS contractors, and other Trustee agencies and their contractors.

J. Facilities

NYSDEC Facilities Available for sample Processing

NYSDEC Saratoga Tree Nursery
2369 Route 50
Saratoga Springs, NY 12866
Telephone: (518) 581-1439

K. Budget

		Funding in $1000 increments and pay periods (in parentheses)[1]							
		FY 2004			FY2005[2]			FY2006	
Personnel									
GS-14 T. Custer									
GS-13 C. Custer									
GS-13 B. Gray									
GS-7 Beckman									
GS-7 Dummer									
GS-7 Houdek									
GS-5 Student (WI)									
GS-5 Student (WI)									
GS-5 Student (WI)									
GS-5 Student (NY)									
A. Karolynish									
W. Yandik									
K. Snyder									
GS-14 Management									
GS-9 Admin									
Travel & Per Diem									
Airline (for all WI Personnel)									
Vehicle & boat - gas And maintenance									
Per Diem (for all WI Personnel)									
Other									
Storage rental									
Vehicles (4) GSA									

Financial information removed as legally privledged.

		Funding in $1000 increments and pay periods (in parentheses) [1]							
		FY 2004			FY2005[2]			FY2006	
Cavity cameras and thermal sensors									
GPS and PDAs									
Misc supplies (cell phones, computer, printer, FAX, books, office Supplies, etc)									
Report Prep / presentations									
UMESC Direct Costs									
USGS overhead (4%)									
Total									

[1] Funds will be transferred from USFWS to USGS. Chemical analytical costs are not included here and will be funded by USFWS directly to a contract laboratory

[2] Work in 2005 is contingent upon 2004 evaluation.

[3] Field crews will involve 2 to 4 teams of two individuals each between mid-April and mid-May, 2004, and 4 to 5 teams of two individuals each between mid-May and early July, 2004.

Financial information removed as legally privledged.

L. Work and Reporting Schedule

	2004/months								2005/months[1]									2006/months									2007/months			
Tasks	2	3	4	5	6	7	8	9-12	1	2	3	4	5	6	7	8	9-12	1	2	3	4	5	6	7	8	9-12	1	2	3	4
Draft Study Plan	■																													
Study Plan Review	■	■																												
Finalize Study Plan		■	■																											
Mobilization – Permitting		■	■								■	■																		
Landowner Contacts		■	■	■							■	■	■																	
Breeding Bird Surveys			■	■	■	■						■	■	■	■															
Nest Checks - Collect Eggs				■	■	■							■	■	■															
Samples submitted for analysis						■	■								■	■														
Demobilization						■	■								■	■														
Prep. Study Plan[2] Amendment 2005								■	■	■																				
Review Study Plan Amendment 2005									■	■	■																			
Final Report Preparation																	■	■	■	■	■	■	■							
Final Report Review[2]																								■	■	■				

[1] Field work in 2005 is contingent upon 2004 evaluation.

[2] A Study Plan amendment will be prepared and provided to the Trustees within 3 months of receipt of chemical analyses.

M. Expected Products

Activity updates are to be provided monthly to the Trustees beginning in April 2004. A Study Plan amendment for Year 2005 will be provided to the Trustees within 3 months of receipt of quality assured analytical chemistry data in electronic format from the Trustees QA Coordinator. This Study Plan amendment will include a summary and discussion of those data and other results from the 2004 efforts, and a proposed study design for 2005. This Study Plan amendment will be subject to further review and approval by the Trustees. Monthly activity updates will be provided to the Trustees until the Study Plan amendment is provided to the Trustees.

The Study Plan amendment for 2005 is to be sent to the U.S. Fish and Wildlife Service New York Field Office, 3817 Luker Road, Cortland, New York 13045, to the attention of Ms. Kathryn Jahn; where possible, electronic copies of the reports are also to be provided to the Trustees (Kathryn_jahn@fws.gov). Monthly activity updates are to be provided to the same address (e-mail preferable).

Results of this study may be published in one or more peer-reviewed scientific journal articles, subject to review and approval by the Trustees.

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ATTACHMENT A. TREE SWALLOW NEST RECORD - HUDSON RIVER STUDY

Colony:			Date:		Weather:	
Investigator:					Study:	
Box#	Nest	Previous	Data	New data		Comments
	Pres?	#eggs	#young	#eggs	#young	

ATTACHMENT B. SURVEY TEAM ACKNOWLEDGEMENT OF WORK PLAN REVIEW

Your signature below indicates that you have read and understood the study plan and associated standard operating procedures.

Name (printed): ____________________ Name (printed): ____________________
Signature: ________________________ Signature: ________________________
Initials: __________________________ Initials: __________________________
Date: ____________________________ Date: ____________________________
Title: ____________________________ Title: ____________________________

Name (printed): ____________________ Name (printed): ____________________
Signature: ________________________ Signature: ________________________
Initials: __________________________ Initials: __________________________
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Title: ____________________________ Title: ____________________________

Name (printed): ____________________ Name (printed): ____________________
Signature: ________________________ Signature: ________________________
Initials: __________________________ Initials: __________________________
Date: ____________________________ Date: ____________________________
Title: ____________________________ Title: ____________________________

ATTACHMENT B CONTINUED. SURVEY TEAM ACKNOWLEDGEMENT OF WORK PLAN REVIEW

Your signature below indicates that you have read and understood the study plan and associated standard operating procedures.

Name (printed): ____________________ Name (printed): ____________________
Signature: ____________________ Signature: ____________________
Initials: ____________________ Initials: ____________________
Date: ____________________ Date: ____________________
Title: ____________________ Title: ____________________

Name (printed): ____________________ Name (printed): ____________________
Signature: ____________________ Signature: ____________________
Initials: ____________________ Initials: ____________________
Date: ____________________ Date: ____________________
Title: ____________________ Title: ____________________

Name (printed): ____________________ Name (printed): ____________________
Signature: ____________________ Signature: ____________________
Initials: ____________________ Initials: ____________________
Date: ____________________ Date: ____________________
Title: ____________________ Title: ____________________

Name (printed): ____________________ Name (printed): ____________________
Signature: ____________________ Signature: ____________________
Initials: ____________________ Initials: ____________________
Date: ____________________ Date: ____________________
Title: ____________________ Title: ____________________

ATTACHMENT C.

CHAIN OF CUSTODY RECORD

Shipped to: ______________________________

______________________________ Fed Ex # ______________

______________________________ Package # ______________

Project Name: Hudson River Avian Study	Project #: USGS 2004 Avian Study	Container Type (e.g., padded egg carrier, cooler, etc.):

Field Processor:
Printed Name and Signature

Sample ID (in format SP YEAR–NUM–E# or N#, such as BK04-010-E1)	Date Collected (MM/DD/YEAR)	Jar size (N/A for whole eggs)	Remarks

Special Instructions/Comments:

Signature	Print Name	Company/Title	Date	Time
Relinquished by:				
Received by:				
Relinquished by:				
Received by:				
Relinquished by:				
Received by:				

Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd.
La Crosse, WI 54603

TECHNICAL OPERATING PROCEDURE

ORIGINAL

PROCEDURE TITLE: Hudson River Avian Study

APPLICABILITY: Any Upper Midwest Environmental Sciences Center (UMESC) or contract personnel responsible for collecting and processing data on the Hudson River.

PRINCIPLE: To describe field surveys, egg and nestling collections, and sample processing.

PRECAUTIONS: Safety

A. Adhere to UMESC vehicle and boat safety regulations.

B. Adhere to Material Safety Data Sheets (MSDS) for chemicals used during sample processing.

PRECAUTIONS: General

When conducting bird studies, disturbance can create biases that affect the gathering and analysis of data and can affect the birds themselves being studied (Gaunt *et al.* 1997). Investigators can cause nest desertion, damage to eggs and young from frightened adults, thermo-damage to eggs or young, mortality from missed feedings or predation, or accidental death from mishandling (Fyfe and Olendorff 1976). During fieldwork and sample collection, all efforts will be made to minimize disturbance to the study species. Many species are believed to be most sensitive to disturbance just prior to and during egg laying (Steenhof 1987). Nest visits during these periods will be avoided as much as practicable, but cannot be completely avoided. Additionally, nest visits will not be performed when weather conditions could prove detrimental to eggs or young (e.g., during a cold, rainy day, or during the middle of a hot, sunny day). Nest visits will be kept as short as possible.

PROCEDURES:

FIELD COLLECTIONS FOR SPOTTED SANDPIPER, BELTED KINGFISHER AND TREE SWALLOW EGGS

A. Equipment, Reagents and other Supplies Needed

1. Plastic compartment boxes and containers
2. Styrofoam or other type of cooler or ice chest
3. Frozen cooler pack(s)
4. Aluminum foil
5. Labels
6. Indelible markers, pencils

7. Egg Data Collection Data Sheets (TS-404.0a)

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B. Egg Sample Collection

1. Eggs will be collected from spotted sandpipers as soon as possible after the clutch is completed and for kingfishers and swallows at or near the estimated hatching date.
2. The best eggs for contaminants analysis are not cracked, since cracking increases variation in percent moisture, and may lead to interference with or contamination of the contents.
3. An egg collection sheet will be completed for each egg collected (TS404.1a). The collected sandpiper and kingfisher egg(s) will be marked with the nest identification (ID) number, wrapped in foil, the foil labeled, and then placed in an egg container. Tree swallow eggs will be placed in a foil-lined box and the box or compartment within the box labeled with the nest ID. Think of the foil as a second skin, which you are using to keep the eggshell together and the contents inside should the egg be cracked in transit. The label on the foil will include the date, species identification, nest number, and collector's initials.
4. The egg container will be put into a secure box, or a cooler with ice packs if needed, and transported to a field lab for processing. At the field laboratory, eggs will be held on ice in coolers or refrigerated, and processed within 48 hours of collection. Ideally eggs will be processed the same day they are collected.
5. Nest ID codes will follow the format "SP YEAR - NUM.". "SP" will be a 2-letter code that designates the species, "YEAR" will be a 2-number code that designates the year, and "NUM" will be a unique three-digit numerical code that corresponds to the nest number (numbers 001 – 999). An example of a nest ID code is SS04-003 or BK04-010.
    - SS = spotted sandpiper
    - BK = belted kingfisher
    - TS = tree swallow

SAMPLE PROCESSING FOR SPOTTED SANDPIPER, TREE SWALLOW AND BELTED KINGFISHER EGGS

A. Equipment, Reagents and other Supplies Needed

1. Aluminum foil
2. Nitrile gloves
3. Glass jars for cleaning & decontamination
4. Tap water
5. Hexane (pesticide/HPLC grade or better)
6. Acetone (pesticide/HPLC grade or better)
7. Labels
8. Indelible marker
9. Chemically clean jars, various sizes
10. Portable field balances
11. Chemically-cleaned dissection equipment, i.e. scissors, forceps, scalpel, etc.
12. Paper towels
13. Scrubby pad

14. Calipers
15. Egg Processing Data Sheets (TS 404.1b)

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B. Sample Processing

1. At the processing facility, the labels on the foil and egg will be double checked against the egg collection data forms to verify the identification code.
2. Set up field laboratory
   - Place a large sheet of aluminum foil on a flat surface.
   - Fill three glass jars with following: clean tap water, hexane and acetone.
   - Select appropriate dissecting tools and decontaminate by washing the equipment in water then rinsing each piece in hexane and then acetone. Lay on a clean piece of foil to dry.
   - Place the balance on a flat surface and verify its accuracy at the beginning of each dissection period. Verification of know-weight weights will be recorded in the instrument log that accompanies each balance.
   - Prepare the Egg Processing Data Sheet (Form TS 404.1b).
   - Prepare labels for sample jars. Include on the label the study name, sample ID, sample type (egg abbreviated as E) as a suffix to the sample ID, date, and PI and processors initials on the label. An example of a label would be:

     Hudson River Study
     SS04-703-E May 30, 2004 or 5/30/04
     RQ & TWC Egg
     - If multiple eggs are collected from one nest they will be designated as E1, E2, etc.
   - Cut an appropriate number of 15 x 15 cm aluminum foil squares upon which the actual egg dissections will be done (see below).
3. Weigh and record the whole egg weight. If debris is present on the egg brush if off prior to weighing. If necessary rinse egg in cool water while gently scrubbing with scrubby pad or sponge. Do not soak the egg. Dry egg prior to weighing.
4. Measure and record the egg length (mm) and width (2 measurements 90° from each other at the widest portion of the egg). Egg volume will be calculated from these measurements.
5. Transfer egg contents to a labeled, chemically-clean jar, purchased in that condition, using the following procedure:
   - Wear nitrile gloves for this part of the procedure. Avoid letting contents run over your hands into the sample jar.
   - Create a catch basin out of the aluminum foil (dull side up) approximately 15 cm on each side by turning edges up and securing the corners. This will catch egg contents in case they spill over the edge of the jar. Use a new piece of foil for each sample. The foil also is a clean place to place your instruments when they are not in use.
   - Tare the chemically-clean empty jar on the balance.
   - Place tared jar in center of aluminum foil.
   - Score equator with serrated blade or scalpel blade. This part takes practice. Cradle the egg in one hand (don't squeeze too tightly!) or set the egg on inside of the jar lid and gently score while rotating the egg. Many light strokes are preferable to a fewer deeper strokes, increasing the evenness of the score and decreasing the possibility of eggshells not separating cleanly or of punching through the shell. Continue to work on your score until you see the membrane, which usually appears gray underneath the

white of the eggshell. When you see the first bit of membrane, place the egg over the jar and finish cutting through membranes with the scalpel. Pour contents into jar, or use the scalpel to gently scrape if that is necessary. Use forceps to remove any shell fragments from the jar. Avoid getting shell dust, or anything else besides the egg contents, in the jar. Try to expose the membrane evenly around the entire egg.

- For swallows, hold the egg vertically with air cell end up. Using scissors cut the top of the eggshell off above the air cell if possible. Pour contents into the jar, or use the scalpel to gently scrape if that is necessary. Use forceps to remove any shell fragments from the jar. Avoid getting shell dust, or anything else besides the egg contents, in the jar.
- Note where the membranes are on the data sheet, as this is important for thickness measurements. For fresh eggs, both membranes often stay with the shell, but as the embryo develops, the inner membrane tends to stick with the chick. If you cannot determine where the membranes are, it often becomes clearer after the eggshell and membranes have dried.
- If the embryo age is >1/2 developed, the embryo will be euthanized by decapitation with a sharp pair of scissors.
- Addled eggs can be full of decomposition by-products, producing gaseous explosions at any weak point in the shell, including where you start your score or where membranes are first exposed. Working with a refrigerated, cool egg reduces this potential, but be prepared for egg explosions.

6. Record the weight of the egg contents. If the balance won't hold a tare, first weigh and record the empty jar weight. Next, record the weight of the egg contents and jar. The sample weight will be calculated by subtraction.
7. Record the stage of embryonic development to the nearest quarter of development. Development options are: no visible development (none), 1/4, 1/2, 3/4, or full term embryo (Powell *et al.* 1998). Also record if the embryo is alive or dead. It may be impossible to determine this for early embryos (<1/4 developed). Note amount of decay or anything else pertinent to the study (cracked shell etc.), and examine for gross deformities, particularly bill deformities such as crossed bills or lack of jaws, but also lack of skull bones, club feet, rotated ankles, dwarfed appendages, microphthalmia, and edema (Gilbertson *et al.* 1991, Fernie *et al.* 2003, Hoffman *et al.* 1998, Kuiken *et al.* 1999, Larson *et al.* 1996, Ludwig *et al.* 1993, Powell *et al.* 1996, Summer *et al.* 1996). Document deformities with photographs whenever possible.
8. Clean and decontaminate dissecting tools in between each egg sample and place a new piece of foil under the sample jar. Use a new scalpel blade as needed. If processing eggs from reference and contaminated locations at the same time, process the reference eggs first and then the contaminated eggs. If soap is needed to clean tools or surfaces, use Alconox.
9. Label the eggshell with the sample ID number and date. Rinse the eggshell halves with cool water and invert on a paper towel to air dry. Store the dried shell halves in a labeled egg carton or other sturdy container.
10. Maintain samples in a cooler (with ice to keep samples cool) or refrigerator, then after each dissection period, place the sample jars in a plastic bag or a box, label bag or box with contents and date, and place in secured freezer. Use security tape if the freezer is not secure. Temperature in the freezer should be checked on at least weekly to verify a -20° C temperature. Weekly temperatures will be recorded in field notebooks.

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FIELD COLLECTIONS FOR BELTED KINGFISHER NESTLINGS

A. Equipment, Reagents and other Supplies Needed

1. Aerated plastic containers
2. Styrofoam or other type of cooler or ice chest
3. Frozen cooler pack(s), or hot water bottle
4. Labels
5. Indelible markers, pencils
6. Nestling collection datasheet (TS 404.1a)

B. Nestling Sample Collection

1. Before collecting samples, place a small amount of grass or other vegetation in the bottom of the container to cushion the nestling. Be sure the container is aerated with air holes punched in the container. Use an indelible ink marker to label each sample container with the Nest ID codes and date.
2. Nest ID codes will follow the format "SP YEAR - NUM.". "SP" will be 2-letter code that designates the species, "YEAR" will be a 2-number code that designates the year, and "NUM" will be a unique three-digit numerical code that corresponds to the nest number (numbers 001 – 999). An example of a nest ID code is BK04-010.
    - BK = belted kingfisher
3. Gently transfer the nestling from the nest into the labeled plastic container.
4. Sample containers must be handled carefully to prevent injury or damage to the nestlings. If samples are stored in a vehicle before being dissected, they should be placed out of the sun and kept cool or warm depending on the season. If the temperature inside the vehicle is uncomfortable to the human occupants then the samples can either be stored in an ice chest with ice packs ("blue ice") to provide a cooler environment or with a hot water bottle to be kept warmer. Samples should be processed as soon as possible or within 2 hours to limit stress on the nestlings and to preserve the stomach contents.

SAMPLE PROCESSING FOR BELTED KINGFISHER NESTLINGS

A. Equipment, Reagents and other Supplies Needed

1. Aluminum foil
2. Nitrile gloves
3. Glass jars for cleaning and decontaminating solutions
4. Tap water
5. Hexane (pesticide/HPLC grade or better)
6. Acetone (pesticide/HPLC grade or better)
7. Labels
8. Indelible marker
9. Chemically clean jars, various sizes
10. Portable field balance
11. Data sheets (TS 404.1c)

12. Chemically-cleaned dissection equipment, i.e. scissors, forceps, scalpel, etc.
13. Paper towels
14. Scrubby pad
15. Calipers

B. Sample Processing

1. At the processing facility, double-check the labels on the young container against the data forms to verify the ID code.
2. Set up the field laboratory
   - Place a large sheet of aluminum foil on a flat surface.
   - Fill three glass jars with following: clean tap water, hexane and acetone.
   - Select appropriate dissecting tools and decontaminate each piece in tap water, then rinse with hexane and then acetone. Lay on a clean piece of foil to dry.
   - Place the balance on a flat surface and verify its accuracy at the beginning of each dissection period. Verification of know-weight weights will be recorded in the instrument log that accompanies each balance.
   - Prepare the nestling data form (Form TS 404.1c)
3. Prepare labels for sample jars. Include sample ID, sample type (carcass, food, etc.) as a suffix to the sample ID, date, and the PI and processors initials on the label. Examples of sample IDs are BK04-002-C for a carcass remainder sample, BK04-002-F for a food sample. An example of a label is:

   Hudson River Study
   BK04-702-L June 23, 2004 or 6/23/04
   CMC & TWC food

   - Cut an appropriate number of 30 x 30 cm aluminum foil squares upon which the actual nestling dissections will be done (see below). Exact size will depend on the size of the bird being dissected but should be sufficiently large to contain the bird and fluids, such as blood.
4. Weigh the young and record weight on data form.
5. Euthanize the nestling by cutting off its head with a sharp pair of scissors (Anonymous 1993). Note: it works well to have one person hold the body with the head stretched out slightly and have the second person wield the scissors. Wear nitrile gloves for this step and for the remainder of the procedure.
6. Dissect out stomach contents as follows:
   - Make an incision in the bird's abdominal wall and open up the body cavity.
   - Remove the stomach using a forceps and small scissors. Slit the stomach open and use a forceps to remove any stomach contents onto a clean piece of tared foil or tared chemically clean jar. Weigh stomach contents and place contents in chemically-clean jar if not already weighed in it.
7. Place the carcass remainder (head, stomach [without contents], and carcass remainder) all together in a labeled, tared sample jar or weigh paper and record the weight. The carcass remainder will either be stored in a chemically clean jar or wrapped in aluminum foil. If the latter, place a label on the outside of the aluminum foil and then place the wrapped carcass remainder in a plastic bag and label the bag.
8. Clean and decontaminate dissecting tools in between each nestling sample and used a new piece of foil for the dissection. If processing nestlings from reference and contaminated locations at the same time, process the nestlings from the reference

location first and the nestlings from the contaminated areas last. If soap is used for cleaning equipment or surfaces use Alconox.

9. Keep the samples in a cooler or refrigerator prior to storage then at the end of each dissection period place the nestling sample jars in a plastic bag or box, label bag or box with contents and date, and place in secured freezer. Use custody seals or locked box if the freezer is not secure. Temperature in the freezer should be checked at least weekly to verify a -20° C temperature.

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REVIEWED BY/APPROVED BY

REVIEWED BY: David A. Kennedy DATE: 17 May 2004
Quality Assurance Officer

APPROVED BY: [signature] (acting) DATE: 17 May 2004
Branch Chief

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Form TS 404.1a. Egg or Nestling Collection Datasheet – Hudson River Avian Study

Collector(s) ______________________________ Page ____ of ____
Name Signature

______________________________ Data Recorder ______________________________
Name Signature Name Signature

Sample ID[1]	Location	Date Collected[2]	Time Collected[3]	Clutch Size	Eggs Warm (yes or no)	Photos Info.	Comments

[1] Format "SP YEAR - NUM-E# or N#" where: "SP" designates the species (SS, spotted sandpiper; BK, belted kingfisher; TS, tree swallow), "YEAR" designates the year (-04 for 2004, -05 for 2005, etc.), "NUM" is a unique three-digit nest number (001 – 999), and E or N indicates whether the sample is an egg or nestling and if it is the first, second, etc. sample of that type from that nest. An example of Sample ID code is BK04-010-E1. [2] In MM/DD/YEAR format, such as 05/04/2004 for May 4, 2004. [3] In 24-hour format, such as 1300 for 1PM

Custody of samples listed above transferred from field collection crew to field laboratory processing crew as follows:

Relinquished by: ______________________________
Signature Print Name Company/Title Date Time

Received by: ______________________________
Signature Print Name Company/Title Date Time

Datasheet checked by: ______________________ Date: __________
Name/Initials

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Form TS 404.1b. Egg Dissection Datasheet - Hudson River Study

Processor(s)____________________ ____________________ Page ____ of ____
Name Signature Name Signature

Date ________ Time ________ Species ________ Jar lot number ________ Balance within limits (yes or no)

Sample ID	Wgt (g) Whole egg wgt.	Measurements (mm) Length1	Length2	Width1	Width2	Embryo devel.[1]	Membrane location[2]	Wgt (g) Egg content wgt.	Comments

Determination of contents weight (if needed):

Sample ID: ____	Sample ID: ____	Sample ID: ____	Sample ID: ____
Weight of jar (g): ____	Weight of jar (g): ____	Weight of jar (g): ____	Weight of jar (g): ____
Weight of jar & contents (g): ____	Weight of jar & contents (g): ____	Weight of jar & contents (g): ____	Weight of jar & contents (g): ____
Weight of contents (g): ____	Weight of contents (g): ____	Weight of contents (g): ____	Weight of contents (g): ____

Calculation of Fresh Weight Conversion Factors for eggs: $CF = \frac{\text{Egg contents weight (g)}}{\text{Calculated egg volume (mm}^3\text{)}} = \frac{\text{Egg contents weight (g)}}{0.51 \times (\text{Average Length})(\text{Average Width})^2}$

Sample ID: ____	Sample ID: ____	Sample ID: ____	Sample ID: ____
Contents weight (g): ____	Contents weight (g): ____	Contents weight (g): ____	Contents weight (g): ____
Average length (mm): ____	Average length (mm): ____	Average length (mm): ____	Average length (mm): ____
Average width (mm): ____	Average width (mm): ____	Average width (mm): ____	Average width (mm): ____
Average width^2 (mm^2): ____	Average width^2 (mm^2): ____	Average width^2 (mm^2): ____	Average width^2 (mm^2): ____
CF: ____	CF: ____	CF: ____	CF: ____

[1] None, ¼, ½, ¾, full term [2] With eggshell (S), With embryo (E)

Datasheet checked by ____________________ Date ________
Name/Initials

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Form TS 404.1c. Nestling Dissection Datasheet - Hudson River Study

Processor(s) ______________________ ______________________ Page ____ of ____
Name Signature Name Signature

Date __________ Time __________ Species __________ Jar lot number __________ Balance within limits (yes or no)

	Weight (g)			
Sample ID	Bird wgt	Stomach contents wgt	Carcass remainder wgt [1]	Comments

Additional Comments:

C (carcass); -F (diet)

[1] Includes head, stomach without contents, carcass remainder

Datasheet checked by ______________________ Date __________
Name/Initials

U.S. DEPARTMENT OF THE INTERIOR
MARCH 3, 1849

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NOAA
U.S. DEPARTMENT OF COMMERCE

DEPARTMENT OF ENVIRONMENTAL CONSERVATION
NEW YORK STATE