AVIAN EXPOSURE AND INJURY STUDY WORKPLAN
for the
St. Louis River/Interlake/Duluth Tar Site
St. Louis River Natural Resource Trustees
September 2002

BACKGROUND AND JUSTIFICATION

This document presents a plan to confirm exposure of avian species to hazardous substances released at the St. Louis River/Interlake/Duluth Tar (SLRIDT) Site, as well as methodologies to evaluate injuries to birds that have been exposed to those substances. This study will be used as described in the Assessment Plan developed for the natural resource damage assessment (NRDA) conducted by the natural resource trustees[1] at the Site. Accordingly, a draft of this workplan was made available for public review before full implementation[2]. The Site is located in Duluth, MN and Superior, WI along the St. Louis River, the second largest tributary to Lake Superior. Industrial activity at the SLRIDT Site began with the Duluth Iron and Steel Co. plant in 1890. This eventually became the Zenith Furnace Company, and later split into the Interlake Iron Company and Duluth Tar and Chemical. Operation of tar and chemical facilities continued until the 1940s, while the iron plant operated until the 1960s. The trustees believe that releases from the Site have contaminated St. Louis River sediments with polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), mercury, arsenic, cadmium, chromium, copper, lead, nickel, and zinc.

Sediment contamination due to the industrial practices described above potentially increases exposure of fish and wildlife to the released hazardous substances through the food web. As the largest estuary along Lake Superior, the lower portion of the St. Louis River is an important source of food and habitat for a wide variety of fish and wildlife, including many avian species. Bird species in the St. Louis River estuary include, but are not limited to the common tern (*Sterna hirundo*) - a threatened species in Minnesota and an endangered species in Wisconsin,

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[1]Trustees include: Minnesota Pollution Control Agency, Minnesota Department of Natural Resources, Fond du Lac Band of Lake Superior Chippewa, The 1854 Authority (representing the Bois Forte, Grand Portage Bands of Lake Superior Chippewa), U S Department of the Interior (Fish and Wildlife Service, Bureau of Indian Affairs), U S Department of Commerce (National Oceanic and Atmospheric Administration).

[2]Collections described in this workplan to confirm exposure to avian species at the SLRIDT Site were started in 2001; these same samples are also intended to be used to evaluate applicable injuries (as described in this workplan) to maintain the efficiency and cost-effectiveness of the assessment.

piping plover (*Charadrius melodus*) - a federally endangered species and an endangered species in Minnesota, ring-billed gull (*Larus delawarensis*), herring gull (*Larus argentatus*), black tern (*Chlidonias niger*), great blue heron (*Ardea herodias*), bald eagle (*Haliaeetus leucocephalus*) - a species of special concern in Minnesota, gyrfalcon (*Falco rusticolus)*, snowy owl (*Nyctea scandiaca*), peregrine falcon (*Falco peregrinus)* - a threatened species in Minnesota, double crested cormorant *(Phalacrocorox auritus),* yellow rail (*Coturnicops noveboracensis*), wood thrush (*Hylocichla mustelina*), golden-winged warbler (*Vermivora chrysoptera*), sedge wren (*Cistothorus platensis*) and a variety of waterfowl. Little is known about exposure and effects of the SLRIDT assessment area contaminants on these and other bird species.

Two primary contaminants of concern at the Site are PAHs and mercury. Even though PAHs can be readily metabolized by birds, these compounds have been detected in bird tissues at contaminated sites (Custer *et al.*, 2001; Custer *et al.*, 2000a). PAHs are known carcinogens and exposure to these compounds may result in adverse effects on survival, growth, metabolism, blood and tissue chemistry, and tumor formation (Eisler, 1987b). Mercury is a known mutagen, teratogen, neurotoxin and carcinogen with a high potential for bioaccumulation and biomagnification in biota. Exposure of birds to mercury may result in adverse effects on growth, development, reproduction, blood and tissue chemistry, metabolism, behavior and histopathology (Eisler, 1987a).

There is insufficient information to confirm that birds from the SLRIDT Site have been exposed to contaminants detected at the Site or that injury has occurred as a result of exposure. However, this type of information is very important not only for establishing exposure and injury, but also to determine ecological and human use service reductions that result from injury to the biological resource. By obtaining exposure, injury, baseline and services information from one study the assessment process can be completed efficiently and at a reasonable cost.

This investigation will determine whether concentrations of PAHs, other organic chemicals, and select metals are present and elevated in birds nesting and feeding at the SLRIDT Site compared to reference sites upstream, as well as whether injuries result from this exposure. Tree swallows (*Tachycineta bicolor*) are used as a representative species to document the extent of contaminant bioavailability and effects to birds with semi-aquatic food habits in and around the Site. These birds readily nest in boxes placed at a site, and feed on mostly emergent aquatic insects in the local vicinity of their nest boxes. Therefore residues in their tissues reflect contaminants available to the birds in an aquatic environment.

OBJECTIVES

The objectives of this study are: 1) to confirm contaminant exposure pathways; 2) to demonstrate the potential for adverse changes in viability to tree swallows at the SLRIDT Site as a result of exposure to the contaminants of concern; 3) to help establish biological baseline ecological services; and 4) to provide information to develop restoration projects at the Site.

CONFIRMATION OF EXPOSURE AND PATHWAY DETERMINATION

Establishing contaminant transport pathways is an important step in conducting any contamination assessment. Potential pathways are dictated by the environment and the natural resources present at the location. Once plausible pathways have been determined, it is necessary to demonstrate that the components making up these pathways have been exposed to the contaminants of concern. Contaminants such as PAHs and mercury are passed from sediments and water to the organisms that inhabit these environments, and can be bioaccumulated to higher trophic level organisms through the food web. Therefore, one plausible exposure pathway at the SLRIDT Site is: sediment as the source of hazardous substances, to benthic organisms that develop into emergent aquatic insects, to birds (both adults and nestlings) that feed on these insects.

In this study, tree swallows are used as a representative bird species. Confirmation of exposure of these birds to PAHs, mercury and other contaminants of concern can be demonstrated by measuring contaminant concentrations in bird tissues, eggs, organisms that make up their diet, and/or by measuring biomarkers that are activated by the contaminants of concern.

PAHs have been detected in bird tissues at other contaminated sites (Custer et al., 2001; Custer et al., 2000a), however the concentration levels do not reflect the full extent of exposure experienced because PAHs can be readily metabolized by birds. As a result of the metabolic process, parent PAH compounds may not be detected. Therefore, it is often necessary to confirm PAH exposure through alternative methods such as measurements of mixed-function oxygenase activity (MFO). MFO activity in birds has been correlated with exposure to petroleum hydrocarbons in the laboratory (Peakall *et al.*, 1987). Three hepatic monooxygenase activities; ethoxyresorufin-O-dealkylase (EROD), benzylosyresorufin-O-dealkylase (BROD), and methoxyresorufin-O-dealkylase (MROD), have been significantly correlated with PAH concentrations in wild lesser scaup carcasses (Custer *et al.*, 2000b). In a study of tree swallows located at a refinery site on the North Platte River, Casper, Wyoming, average hepatic monooxygenase activities in tree swallow livers were more than nine times higher than in tree swallow livers from birds located at a nearby uncontaminated reference site (Custer et al., 2001).

Exposure and pathway information obtained from this study will be combined with results from other studies conducted at the SLRIDT Site, such as the trustees' fish study (Trustees, 2002), to further characterize contaminant pathways through the environment. This may include studies conducted on surface water, sediments, ground water, fish, insects and other appropriate pathway components.

INJURY DETERMINATION

Exposure of organisms to PAHs, mercury and other contaminants of concern can result in adverse biological effects such as impaired reproductive success, increased tumor and lesion formation, cancer, genetic mutations and mortality. A cost effective study design can take advantage of the same organisms used in confirming contaminant exposure for the purpose of injury identification.

U. S. Department of the Interior NRDA regulations (43 CFR §11.62(f)) provide definitions used to assess injuries to biological resources. An injury to a biological resource has resulted from the discharge of a hazardous substance if concentration of the substance is sufficient to :

1) Exceed action or tolerance levels established under section 402 of the Food, Drug and Cosmetic Act, 21 U.S.C. 342 in edible portions of organisms;

2) Exceed levels for which an appropriate State health agency has issued directives to limit or ban consumption of such organism (43 CFR §11.62(f)(1)(iii); or

3) Cause the biological resource or its offspring to have undergone at least one of the following adverse changes in viability: death, disease, behavioral abnormalities, cancer, genetic mutations, physiological malfunctions (including malfunctions in reproduction), or physical deformations (43 CFR §11.62(f)(1)(i)).

Site-specific evaluations of the relationship between the above criteria and hazardous substances released at the Site have not been fully implemented. Injury determination, as proposed by this study, will focus on adverse changes in viability of birds, including but not limited to, physiological malfunctions and genetic mutations. Other studies have used flow cytometry to detect chromosome damage in wildlife exposed to certain environmental contaminants (Bickham, 1990; Custer *et al.*, 1994), and have demonstrated a direct correlation between PAH concentrations in tissues and somatic chromosomal damage in blood (Custer et al., 2000b). Measures of oxidative stress in livers of birds have been highly correlated with mercury concentrations (Custer *et al.*, 1997). These bioindicator measurement methods are detailed in later sections.

SERVICE LOSS EVALUATION AND RESTORATION POTENTIAL

Natural resources provide ecological and human use services such as boating, swimming, bird watching, habitat for fish and wildlife, quality food resources and other services. However, injured natural resources may result in lost services that would have been provided had the release of hazardous substances not occurred. Determining the extent of lost services at the assessment site, and appropriate restoration alternatives associated with those losses, is dependent on acquiring insight into the welfare of the natural resources without the adverse effects of the hazardous substances. To acquire this information, the same type of bird data collected from the assessment site is also collected from a reference (control) location. Comparison of the reference and assessment site data contributes to determining the extent of lost services (both ecological and human use) at the assessment site.

Data from the reference area provides baseline information that helps to assess the restoration potential for the assessment area. From the baseline information, restoration plans can be developed that focus on the assessment site's potential for providing viable natural resources. Additionally, baseline information serves as a reference point for monitoring the assessment area to determine the effectiveness of restoration activities. Integration of these restoration projects into remedial actions at a site contribute to efficient natural resource damage assessments. Therefore, this study will provide valuable information on exposure, baseline conditions, injury, and restoration potential at the SLRIDT Site at a reasonable cost.

The following table describes categories that are evaluated in this study with an overview of the associated methods used to establish exposure or injury. The methods selected are those that the trustees have determined to be the most applicable and provide the best information for the SLRIDT Site. The table also provides information on the sensitivity of the selected method and potential restoration considerations that may be associated with injuries.

Biological responses in birds, injury determination methods and potential restoration considerations:

Biological Response	Indicator of Response	Testing Method	Tissues Examined	Sensitivity	Potential Restoration Consideration
Physiological malfunctions 43 CFR 11.62 (f)	Biochemical changes	Oxidative Stress GSH TSH PBSH	Liver	Mercury	Enhance quality of food resources. Enhance breeding habitat. Enhance birdwatching opportunities.
		EROD	Liver	PAHs OCs Dioxins	
	Reproductive success	Mayfield Analysis Hatching success Egg fertility Survival of young			
Genetic Mutations 43 CFR 11.62 (f)	Chromosome changes	Chromosome Damage CV DNA	Blood - flow cytometry	Mercury PAHs	Enhance quality of food resources. Enhance breeding habitat.
Accumulation of Contaminants	Tissue concentrations	Mercury - cold vapor AA PAHs - GC/MS	Bird tissue Prey items Eggs	Mercury PAHs	Enhance quality of food resources.

GSH = glutathione
TSH = total thiol
PBSH = protein-bound thiol
EROD = ethoxyresorufin-O-dealkylase
CV = coefficient of variation

METHODS

The following sampling protocols have been developed to accomplish study objectives. This study focuses on the exposure of birds to mercury and PAHs and the resulting effects from such exposure.

Sample Collections

A total of approximately 70-100 tree swallow boxes are installed in and around the SLRIDT Site and at one or two reference locations. Two areas at the Site, Stryker Bay and Keene Creek Embayment/Slip 7, have been selected as preferred swallow habitat. Site access was obtained in 2000, and some nest boxes were put in place to acclimate swallows to the area. In addition, a location immediately upstream from the Site, Kingsbury Bay, was selected for use in 2001 and 2002 to evaluate the extent of exposure. The reference site, North Bay, was selected for 2002 collections. Approximately 20 nest boxes are placed at each Site location; approximately 30 nest boxes are at the reference location.

Each nest box is visited approximately once per week with visits increasing in frequency as the nesting chronology proceeds. Recorded information includes: date, extent of nest development, number of eggs, number of young, and any other pertinent observations. Samples of 2-3 eggs and/or just-hatched eggs (pippers) and two sibling 12-13 day-old nestling are collected from 5-10 boxes at each site. The exact number of eggs collected per nest depends on sample mass with a goal of 3g + 1g for the 2-3 pippers. Eggs and nestlings are collected under appropriate federal and state permits from the first 5-10 clutches that are initiated at each site with later clutches possibly utilized for temporal comparisons. If late clutches are sampled, numbers of samples will be greater than indicated in this workplan. Each collected egg and nestling sample is given a unique numerical identifier in the field. All identification numbers are recorded on data sheets. The field team also documents its sampling activities and field measurements.

Diet (prey items) of tree swallows are also collected from nestlings by use of throat ligatures applied to chicks older than 6-7 days of age. Ligatures are placed around the throat of all nestlings within the brood and left on for one hour (Orians, 1966). Food boli from 5 or more nests at each site are pooled, placed in chemically clean jars, and frozen. A sub sample of the remainder of the bolus samples is analyzed for species identification.

Sample Handling and Preparation

Egg contents are pooled for each clutch, placed in a chemically clean jar, and frozen. Embryos and nestlings are euthanized by decapitation. Immediately after decapitation of the nestling, blood is collected using heparinized capillary tubes and aspirated into a cryotube containing freezing media (Ham's F10 media with 18% fetal calf serum and 10% glycerine). Each cryotube is frozen in liquid nitrogen and later transferred to an ultra cold freezer where it is stored at -80ºC until analysis by flow cytometry (Custer et al., 1997). Nestling livers are excised, and contents in the upper gastrointestinal (GI) tract removed. Immediately after blood collection, a portion

(<0.5g) of the nestling liver is placed into cryotubes, quick frozen in liquid nitrogen, and then stored in a ultra cold freezer at -80ºC for analysis of four measurements of oxidative stress (n = 23 in 2001; n=34 in 2002) (Custer et al., 1997). Another portion of the liver is removed from chicks immediately after blood collection, weighed (±0.1 g) and about 0.3 g from one chick per brood is placed into a cryotube for measurement of monooxygenase activity (n = 23 in 2001; n=34 in 2002). A few drops of glycerin are added to the cryotube and immediately placed into liquid nitrogen. The cryotubes are later transferred from the liquid nitrogen to an ultracold freezer at -80ºC for storage until processing for EROD induction.

In addition to nestling diet samples, GI tract contents are placed in individual chemically clean jars and frozen. Diet samples in the stomachs of nestlings from 5 or more nests at each site are pooled, placed in chemically clean jars, and frozen.

Chemical/Biomarker Analyses

Chemical Measurements - Samples are analyzed for PAHs, alipahtic hydrocarbons (AHs), other organic compounds, and metals as follows:

Sample Type	**Number of Samples by Analytical Parameter**[3]							
	PAH/AH Scan		**Metals Scan**		**OC Scan**[4]		**Dioxin Scan**[4]	
	2001	2002	2001	2002	2001	2002	2001	2002
Eggs	-	-	13	34	-	-	-	-
Livers	-	-	23	34	-	-	-	-
Chicks	23	34	23	34	23	34	0	4
Diet	5	8	2	4	-	-	-	
Totals	28	42	61	106	23	34	0	4

[3]Number of samples analyzed in 2002 may be greater than those indicated if late-nesting samples are collected for temporal comparisons.

[4]OC and dioxin scans are performed to determine the role of these contaminants in inducing EROD activity.

Chemical analyses are completed according to the standard operating procedures of the U. S. Fish and Wildlife Service Patuxent Analytical Control Facility (PACF) contract laboratory. PACF maintains a rigorous QA/QC program for sample analysis and selects contract laboratories based on their ability to meet the QA/QC requirements to assure valid analyses.

Contaminants measured in tree swallow chicks represent local exposure when chemical burdens in eggs are factored out (Custer *et al.*, 1995). Accumulation rates (μg/day) of contaminants in chicks can be compared to reference and extremely contaminated locations (e.g., Indiana Harbor, Indiana). For example, 12-day-old barn swallow chicks accumulated 30 μg chromium/day at Indiana Harbor, IN and only 0.4 μg chromium/day at a reference location (D. Sparks in preparation). Analyses of prey items collected by tree swallow adults and fed to chicks will indicate bioavailability of contaminants through the aquatic invertebrate food chain.

Bioindicator Measurements -Chick blood samples are obtained at the time of other tissue collections and analyzed for flow cytometry (n = 23 in 2001; n=34 in 2002). Flow cytometry can be used to detect chromosome damage in wildlife exposed to certain environmental contaminants (Bickham, 1990; Custer et al., 1994). Because mercury and PAHs are known to damage chromosomes, blood from tree swallow chicks exposed to increased mercury and PAH concentrations at the Site may show a difference in chromosome damage compared to chicks at the reference location.
Measures of oxidative stress in livers of birds are highly correlated with mercury concentrations (Custer et al., 1997). Therefore, oxidative stress in tree swallow chicks may result from exposure to mercury in the environment.

Hepatic microsomes are prepared from homogenates of thawed liver samples by differential centrifugation. EROD activity is assayed by the methods of Burke and Mayer (Burke *et al.*, 1974; 1983) as adapted to a fluorescence microwell plate scanner (Melancon, 1996); activities are calculated as pmol product/min/mg microsomal protein. Induction of hepatic monooxygenase activity following exposure to PAHs has been documented in birds in both the laboratory (Jellinck *et al.*, 1973; Peakall *et al.*, 1989; Trust *et al.*, 1994; Walters *et al.*, 1987) and the field (Custer et al., 2000b). Although PAHs are rapidly metabolized in birds (Naf *et al.*, 1992), they have been detected in bird tissues in relation to sources of petroleum (Custer et al., 2000a) and combustion (Mineau *et al.*, 1984).

Biological Effects

The potential effects of contaminants to nesting birds at the SLRIDT Site are addressed using several approaches. First, tree swallow reproduction in relation to contaminants exposure is assessed in both 2001 and 2002. This investigative aspect consists of a collection of a sample egg from selected nests, and the subsequent evaluation of the hatching success of the remaining eggs. Logistic regression techniques are used to correlate levels of chemical residues in eggs

that are collected with the hatching success of sibling eggs (reference statistical analyses, below). The sample egg method has been useful in documenting the effects of contaminants on breeding birds at other locations. For example, the sample egg method demonstrated a relationship between reproductive success and DDE concentrations in black-crowned night-heron eggs (Custer *et al.*, 1983) and black skimmer eggs (Custer *et al.*, 1987). Second, reproductive success at each tree swallow site is quantified using the Mayfield method (Mayfield, 1961; 1975) and compared among sites according to Hensler and Nicols (Hensler *et al.*, 1981). The Mayfield analysis is a method to statistically compare reproductive success measures by quantifying the probability that an egg, nestling, or nest attempt will survive each day. The probabilities are calculated for each site and the sites or years can then be compared. Third, egg and tissue concentrations for swallows are compared to values which are considered elevated or toxic to other species. For example, mercury concentrations > 0.5 ppm wet weight in eggs were associated with reduced reproduction in ring-necked pheasants (Fimreite, 1971). Finally, bioindicator measurements in nesting tree swallows document both exposure and avian physiological and genetic responses to contaminants at the Site which may contribute to other biological effects.

Data Analysis

Statistical Analyses -Preliminary exploratory analyses are performed to verify the data and identify unduly influential observations that may be important outliers. Contaminant levels and bioindicators in biota are compared among locations using one-way analysis of variance; conformation of the data to model assumptions is checked, and remedial measures are implemented where necessary (Neter *et al.*, 1990). Comparisons among the tree swallow sites is made using Dunnett's multiple comparison procedure (Dunnett, 1980). Linear correlation, using Pearson correlation coefficients, is used to evaluate the response of bioindicators to PAH, AH, and metal concentrations. Reproductive success is quantified using the Mayfield method (Mayfield, 1961; 1975) and compared among sites according to Hensler and Nicols (Hensler et al., 1981). The response of hatching success to contaminant concentrations is modeled using logistic regression (Hosmer *et al.*, 1989). This statistical approach was chosen because egg success is a binary dependent variable (i.e., either an egg hatched or it did not). Logistic regression allows multiple variables to be evaluated simultaneously in relation to the dependent variable (i.e., egg success). Logistic regression is also used to fit a curvilinear response function between each variable and egg success. The Hosmer and Lemeshow Goodness-of-Fit test (1989) is used to determine whether the data adequately fit the logistic function; a P value >0.05 indicates an adequate fit. Type I error of 0.05 is used for all statistical analyses.

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REFERENCES

Bickham, J. W. (1990). Flow Cytometry as a Technique to Monitor the Effects of Environmental Genotoxins on Wildlife Populations. In Situ Evaluations of Biological Hazards of Environmental Pollutants. S. S. Sandhu, W. R. Lower, F. J. deSerres, W. A. Suk and R. R. Tice. NY, Plenum Publ. Corp**:** 97-108.

Burke, M. D. and R. T. Mayer (1974). Ethoxyresorufin: Direct Fluorimetric Assay of a Microsomal O-Dealkylation Which Is Preferentially Inducible by 3-Methylcholanthrene. *Drug Metab. Dispos.* **2**: 583-588.

Burke, M. D. and R. T. Mayer (1983). Differential Effects of Phenobarbitone and 3-Methylcholanthrene Induction on the Hepatic Microsomal Metabolism and Cytochrome P-450-Binding of Phenoxazone and a Homologous Series of Its N-Alkyl Ethers (Alkoxy Resorufins). *Chem-Biol. Interact.* **45**: 243-258.

Custer, T. W.; J. W. Bickham; T. B. Lyne; T. Lewis; L. A. Ruedas; C. M. Custer and M. J. Melancon (1994). Flow Cytometry for Monitoring Contaminant Exposure in Black-Crowned Night-Herons. *Arch. Environ. Contam. Toxicol.* **27**: 176-179.

Custer, T. W. and C. M. Custer (1995). Transfer and Accumulation of Organochlorines from Black-Crowned Night-Heron Eggs to Chicks. *Environmental Toxicology and Chemistry* **14**: 533-536.

Custer, T. W.; C. M. Custer; K. Dickerson; K. Allen; M. J. Melancon and L. J. Schmidt (2001). Polycyclic Aromatic Hydrocarbons, Aliphatic Hydrocarbons, Trace Elements and Monooxygenase Activity in Birds Nesting on the North Platte River, Casper, Wyoming, USA. *Environmental Toxicology and Chemistry* **20**(3): 624-631.

Custer, T. W.; C. M. Custer; R. K. Hines and D. W. Sparks (2000a). Trace Elements, Organochlorines, Polycyclic Aromatic Hydrocarbons, Dioxins, and Furans in Lesser Scaup Wintering on the Indiana Harbor Canal. *Environmental Pollution* **110**: 469-482.

Custer, T. W.; C. M. Custer; R. K. Hines; D. W. Sparks; M. J. Melancon; D. J. Hoffman; J. W. Bickham and J. K. Wickliffe (2000b). Mixed-Function Oxygenases, Oxidative Stress, and Chromosomal Damage Measured in Lesser Scaup Wintering on the Indiana Harbor Canal. *Arch. Environ. Contam. Toxicol.* **38**: 522-529.

Custer, T. W.; G. L. Hensler and T. E. Kaiser (1983). Clutch Size, Reproductive Success, and Organochlorine Contaminants in Atlantic Coast Black-Crowned Night-Herons. *Auk* **100**: 699-710.

Custer, T. W.; R. H. Hines; M. J. Melancon; D. J. Hoffman; J. W. Bickham; J. W. Martin and D. Henshel (1997). Contaminant Concentrations and Biomarker Response in Great Blue Heron Eggs from 10 Colonies on the Upper Mississippi River, USA. *Environmental Toxicology and Chemistry* **16**: 260-271.

Custer, T. W. and C. A. Mitchell (1987). Organochlorine Contaminants and Reproductive Success of Black Skimmers in South Texas. *Journal of Field Ornithology* **58**: 480-489.

Dunnett, C. W. (1980). Pairwise Multiple Comparisions in the Homogeneous Various, Unequal Sample Size Case. *J. Amer. Stat. Assoc.* **75**: 789-795.

Eisler, R. (1987a). Mercury Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. U. S. Department of the Interior; Fish and Wildlife Service**:** pps. 90.

Eisler, R. (1987b). Polycyclic Aromatic Hydrocarbon Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. U. S. Department of the Interior; U. S. Fish and Wildlife Service**:** pps. 81.

Fimreite, N. (1971). Effects of Dietary Methylmercury on Ringnecked Pheasants. *Can. Wildl. Ser. Occasion. Pap. 9*.

Hensler, G. L. and J. D. Nichols (1981). The Mayfield Methods of Estimating Nesting Success: A Model, Estimators and Simulation Results. *Wilson Bull.* **93**: 42-53.

Hosmer, D. W. and S. Lemeshow (1989). Applied Logistic Regression. New York, NY, John Wiley and Sons.

Jellinck, P. H. and G. Smith (1973). Aryl Hydrolase Induction in the Chick Embryo by Polycyclic Hydrocarbons. *Biochim. Biophys. Acta* **304**: 520-525.

Mayfield, H. (1961). Nesting Success Calculated from Exposure. *Wilson Bull.* **73**: 255-261.

Mayfield, H. (1975). Suggestions for Calculating Nest Success. *Wilson Bull.* **87**: 456-466.

Melancon, M. J. (1996). Development of Cytochromes P450 in Avian Species as a Biomarker for Environmental Contaminant Exposure and Effect. Procedures and Baseline Values. Environmental Toxicology and Risk Assessment: Biomarkers and Risk Assessment. Vol 5 Astm Stp 1306. D. A. Bengston and D. S. Henshel. Philadelphia, PA, American Society for Testing and Materials. **5:**95-108.

Mineau, P.; G. A. Fox; R. J. Norstrom; D. V. Weseloh; D. J. Hatllett and J. A. Ellenton (1984). Using the Herring Gull to Monitor Levels and Effects of Organochlorine Contamination in the Canadian Great Lakes. Toxic Contaminants in the Great Lakes. J. O. Nriagu and M. S. Simmons. New York, John Wiley & Sons**:** 425-452.

Naf, C. D.; D. Broman and B. Brunstrom (1992). Distribution and Metabolism of Polycyclic Aromatic Hydrocarbons (Pahs) Injected into Eggs of Chicken (*Gallus Domesticus*) and Common Eider Duck (*Somateria Mollissima*). *Environmental Toxicology and Chemistry* **11**: 1653-1660.

Neter, J.; W. Wasserman and M. N. Kutner (1990). Applied Linear Statistical Models; Regression, Analysis of Variance, and Esperimental Design. Homewood, Illinois, Irwin Press.

Orians, G. H. (1966). Food of Nestling Yellow-Headed Blackbirds, Caribou Parklands, British Columbia. *Condor* **68**: 321-337.

Peakall, D. B.; D. A. Jeffrey and D. Boersma (1987). Mixed-Function Oxidase Activity in Seabirds and Its Relationship to Oil Pollution. *Comp. Biochem. Physiol.* **88C**: 151-154.

Peakall, D. B.; R. J. Norstrom; D. A. Jeffrey and F. A. Leighton (1989). Induction of Hepatic Mixed Function Oxidases in the Herring Gull (*Larus Argentatus*) by Prudoe Bay Crude Oil and Its Fractions. *Comp. Biochem. Physiol.* **94C**: 461-463.

Trust, K. A.; A. Fairbrother and M. J. Hooper (1994). Effects of 7,12-Dimethylbenz[a]Anthracene on Immune Function and Mixed-Function Oxygenase Activity in the European Starling. *Environmental Toxicology and Chemistry* **13**: 821-830.

Trustees (2002). Fish Exposure and Injury Study for the St. Louis River/Interlake/Duluth Tar Site. St. Louis River Natural Resource Trustees.

Walters, P.; S. Kahn; P. J. O'Brien; J. F. Payne and A. D. Rahimtula (1987). Effectiveness of Prudhoe Bay Crude Oil and Its Aliphatic, Aromatic and Heterocyclic Fractions in Inducing Mortality and Aryl Hydrocarbon Hydroxylase in Chick Embryo in Ovo. *Arch. Toxicol.* **60**: 454-459.