FISH 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 fish to hazardous substances released at the St. Louis River/Interlake/Duluth Tar (SLRIDT) Site, as well as methodologies to evaluate injuries to fishery resources resulting from this exposure. This study is part of the Assessment Plan developed for the natural resource damage assessment (NRDA) conducted by the natural resource trustees[1]. 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. The lower portion of the St. Louis River is the largest estuary along Lake Superior and as such provides important habitat for a wide variety of fish and wildlife. More than 50 native species of fish are supported by the River, including lake sturgeon (*Acipenser fulvescens*) - a federal and

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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]Fish collections described in this workplan to confirm exposure at the SLRIDT Site were completed 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.

state species of concern, walleye (*Stizostedion vitreum*), black crappie (*Pomoxis nigromaculatus*), carp (*Cyprinus carpio*), muskellunge (*Esox masquinongy*), northern pike (*Esox lucius*), smallmouth bass (*Micropterus dolomieu*), channel catfish (*Ictalurus punctatus*), and white sucker (*Catostomus commersoni*). Fish consumption advisories due to PCB and mercury concentrations have been issued by both Minnesota and Wisconsin for the St. Louis River. Minnesota advisories include walleye, black crappie, carp, lake sturgeon, northern pike, channel catfish, sunfish, white sucker, yellow perch and smallmouth bass due to mercury contamination (MDH, 2002).

Two primary contaminants of concern at the Site are PAHs and mercury. PAHs have been implicated in the development of liver carcinogenesis (Baumann *et al.*, 1991; Malins *et al.*, 1987; Vogelbein *et al.*, 1990) and a cause and effect relationship between PAHs and liver tumors or preneoplastic lesions in fish has been established by experimental studies (Metcalfe *et al.*, 1988; Schiewe *et al.*, 1991). Additional evidence linking sediment PAHs with liver tumors was observed with bottom-feeding brown bullheads (*Ameiurus nebulosus*) from the Black River in Ohio. The study showed that liver tumor prevalence correlated with increased and decreased changes in sediment PAH concentrations (Baumann *et al.*, 1998). In summarizing several Laurentian Great Lakes surveys, Black and Baumann (1991) discovered higher oral and cutaneous tumor prevalence in brown bullheads located in PAH contaminated tributaries relative to uncontaminated creeks or lakes.

Mercury is a known mutagen, teratogen, neurotoxin, and carcinogen with a high potential for bioaccumulation and biomagnification in aquatic biota. The majority of mercury (>90%) detected in fish is methylmercury (Grieb *et al.*, 1990; Hildebrand *et al.*, 1980; Westoo, 1973) which is the most toxic and most readily accumulated form of mercury (Clarkson, 1973). Relatively low concentrations of mercury in fish can adversely affect their reproduction, growth, behavior, metabolism, blood chemistry, osmoregulation and oxygen exchange (Eisler, 1987).

There is insufficient information to confirm that fish 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. This information can be obtained efficiently and at reasonable cost from one study, provided the study is designed with the above goals in mind.

OBJECTIVES

The objectives of this study are: 1) to confirm contaminant exposure pathways; 2) to document adverse changes in viability to fish 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. One exposure pathway being considered at the SLRIDT Site is the release of hazardous substances to Site sediments and surface water, bioaccumulation of the substances by lower trophic level benthic and pelagic organisms, and further bioaccumulation of hazardous substances by fish.

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. In this study the pathway component being examined is fish. Confirmation of exposure can be established by measuring contaminant concentrations in fish tissue and/or by measuring biomarkers that are activated by the contaminants of concern. Target fish species include white sucker (*Catostomus commersoni*), northern pike (*Esox lucius*), shorthead redhorse (*Moxostoma macrolepidotum*), yellow perch (*Perca flavescens*), black crappie (*Promoxis nigromaculatus*) and rock bass (*Ambloplites rupestris*) as representative fish species. Species selection was based on food web trophic position, expected abundance at the Site, and the territorial nature of the species.

Exposure and pathway information obtained from this study will be combined with results from other studies such as the trustees' avian study (Trustees, 2002) conducted at the SLRIDT Site 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 and mercury can result in adverse biological effects such as impaired reproductive success, behavioral changes, 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 developed. Injury determination will be based on overall fish health assessment, fish tissue concentrations of contaminants, tumor formation and biomarker measurements. Adverse changes in viability of fish will focus on: disease, cancer, physiological malfunctions, and physical deformations.

SERVICE LOSS EVALUATION AND RESTORATION POTENTIAL

Natural resources provide ecological and human use services such as boating, swimming, fishing, 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 (e.g., fish) without the adverse effects of the hazardous substances. To acquire this information, the same type of fish 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) associated with this resource at the assessment site.

Data from the reference area provides baseline information that helps to assess the restoration potential for the assessment area. In this study, overall fish health, fish tissue contaminant concentrations and fish biomarker measurements from a reference site provide baseline conditions for comparisons with 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 fishery resources at 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 fish, injury determination methods and potential restoration considerations:

Biological Response	Indicator of Response	Testing Method	Tissues Examined	Sensitivity	Potential Restoration Consideration
Disease 43 CFR 11.62 § (f)(4)(ii)	Incidence and severity of viral, bacterial and parasitic infections, fin erosion	Necropsy-based fish health assessment	Whole fish (internal and external)	Overall organism health, may indicate contaminant stress	Improve recreational fishing opportunities. Enhance fish habitat. Watershed-based protection of existing fish habitat.
Cancer 43 CFR 11.62 § (f)(4)(iv)	Fish neoplasm (skin and liver tumors and pre-tumors)*	Histopathology (cellular level) and necropsy-based fish health assessment	Liver (cellular level) and whole fish	Overall organism health and contaminant stress	Improve recreational fishing opportunities. Enhance fish habitat. Watershed-based protection of existing fish habitat.
Physiological malfunctions 43 CFR 11.62 § (f)(4)(v)	Biochemical changes	EROD DNA Adducts	Liver	PAHs, PCBs, dioxins, and furans (EROD) PAH-specific (DNA Adducts)	Enhance fish habitat. Watershed-based protection of existing fish habitat.
Physical deformation** 43 CFR 11.62 § (f)(4)(vi)	Deformities in embryos, lesions in various organs	Necropy-based fish health assessment, histopathology	Whole fish	Overall organism health - may indicate contaminant stress	Improve recreational fishing opportunities. Enhance fish habitat. Watershed-based protection of existing fish habitat.
Fish Tissue Concentrations 43 CFR 11.62 § (f)	Exceed FDA action or tolerance levels in edible portions of fish. Exceed State fish consumption levels	Mercury - cold vapor AA PAHs - GC/MS	Whole fish	Methods specific to mercury and individual PAH compounds	Improve recreational fishing opportunities. Enhance fish habitat. Watershed-based protection of existing fish habitat.

* Neoplasms are characterized by relatively autonomous growth of abnormal cells that by proliferation infiltrate, press upon, or invade healthy tissue thereby causing destruction of cells, interference with physiological functions, or death of the organism. Types - liver neoplasia, skin neoplasia (confirmed by histological procedures and may also include special staining techniques for specific tissue components, ultra-structural examination using electron microscopy to identify cell origin and to rule out or confirm viral, protozoan, or other causal agents.

**Physical deformation - overt external malformations (missing eyes or small eyes), skeletal deformities, internal whole organ and soft tissue malformation to brain, heart, liver, kidney and other organs as well as soft tissues of gastrointestinal tract and vascular system, histopathological lesions.

METHODS

The following sampling protocols have been developed to accomplish study objectives. This study focuses on exposure of fish to mercury and PAHs as the primary Site contaminants of concern, however additional analyses for other contaminants may be conducted.

SAMPLE COLLECTION

Fish are collected in the Keene Creek Embayment/Slip 7 and in Stryker Bay located at the SLRIDT Site, and at North Bay, a reference area located approximately 8-10 miles upriver. Fish may also be collected from other areas near the site, such as Kingsbury Bay[3], an embayment immediately upstream from Stryker Bay, to evaluate the extent of exposure. Fish samples are collected during late August through September in 2001 and 2002 using electrofishing methods.

Pelagic Fish Species

Yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) representing pelagic fish species are collected from each site. Collection goals for yellow perch are to collect four individual fish[4] from each of five different size ranges, for a total of 20 yellow perch per site. Size ranges collected are dependant on availability at each site (ie. not all size groups may be found at all sites). For young-of-the-year perch, individual fish may not have adequate mass for chemical analyses, in which case fish will be composited to comprise enough mass for one sample. The collection goal for northern pike is up to 10 - 20 individual adult fish per site. Yellow perch and northern pike are important food web components that are representative of a valuable recreational fishery resource in the area.

Benthic Fish Species

Target benthic fish species include white sucker (*Catostomus commersoni*) and shorthead redhorse (*Moxostoma macrolepidotum*). Collection goals are 10 - 20 adult fish from each site. These fish species are important food web components directly associated with sediments.

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[3]Fish were collected from Kingsbury Bay during the 2002 sampling season.

[4]Composite samples were collected during the 2001 sampling season, ie. a goal of 3 composites consisting of up to five individual fish for each of at least three size classes.

SAMPLE HANDLING AND PREPARATION

Collected fish are placed in live-wells until processing. Each individual is measured for length and weight, and then euthanized. Scales from each fish are collected for age determination[5].

Yellow Perch (All)

Yellow perch samples are placed in plastic bags[6] to comprise one sample. Each sample is labeled with an identification number, weight and length information. Samples are kept on ice until frozen for chemical analysis.

Adult Northern Pike, White Sucker, Shorthead Redhorse

Adult fish are processed within 1 to 6 hours of collection. A necropsy-based fish health assessment is performed applying the U.S. Geological Survey Biomonitoring of Environmental Status and Trends (BEST) Program protocols (Schmitt *et al.*, 1999). An external and internal examination is conducted to detect grossly observable lesions involving the skin, gills, and tissues within the oral cavity. Any unusual features that are grossly visible are reported. Gonads are examined and classified as immature, male, or female.

Gallbladder condition is examined for degree of fullness prior to bile collection. A bile sample (up to 200µL) is removed by puncturing the gall bladder with a 5mL syringe, transferred to a microvial situated in an 4mL amber vial and stored at -20ºC until analysis for PAH metabolites. Bile color is also noted on the health assessment sheet.

After bile collection, the liver is excised and examined for gross lesions. If there are any lesions a 3 or 4 mm thickness with both nodule and normal tissue present is saved and preserved as indicated below for histology. Duplicate samples[7] of approximately 0.4 g section of liver are flash frozen in liquid nitrogen and stored at -80ºC for EROD activity analysis. An additional 0.5g portion of the liver is flash frozen in liquid nitrogen and stored at -80ºC for analysis of DNA adducts. The remainder of the liver is placed in a 125 mL polyethylene bottle in Dietrich's fixative for histological analysis. The preferable ratio of fixative to liver is 20:1. The bottle is then lightly shaken to ensure that the liver is fully engulfed in fixative.

Remaining internal organs are placed back into the abdominal cavity of the fish. Each fish is wrapped in aluminum foil, labeled, and placed in a plastic bag. Samples are kept on ice until frozen for subsequent chemical analysis.

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[5]Scales were not collected from yellow perch in 2001.

[6]Fish from the 2001 sampling season were wrapped in aluminum foil.

[7]Duplicate samples were not collected in 2001.

CHEMICAL/BIOMARKER ANALYSES

Yellow Perch

Concentrations of total mercury are determined in whole body yellow perch fish samples following quality assurance and control protocols specified by the Patuxent Analytical Control Facility of the U.S. Fish and Wildlife Service. The facility maintains a rigorous QA/QC program for sample analysis and selects contract laboratories based on their ability to meet the QA/QC requirements.

Adult Northern Pike, White Sucker, Shorthead Redhorse

Whole fish are analyzed for mercury and PAH concentrations following quality assurance and control protocols specified by the Patuxent Analytical Control Facility of the U.S. Fish & Wildlife Service. The facility maintains a rigorous QA/QC program for sample analysis and selects contract laboratories based on their ability to meet the QA/QC requirements.

Because fish have the ability to metabolize PAH compounds, these chemicals may not bioaccumulate to full potential and concentrations detected in fish tissue can be misleading. Therefore, additional analyses of biomarkers signaling PAH exposure is necessary to fully determine contaminant pathways, extent of exposure, and injury due to these chemicals. Three commonly applied and recommended biomarkers for detection of PAH contamination in fish are: fluorescence analysis of PAH metabolites in bile, ethoxyresorufin-*O*-deethlyase (EROD) activity in liver estimating cytochrome P4501A (CYP1A) induction, and formation of DNA adducts in liver (Stagg, 1998). Responses in these biomarkers have been correlated to more adverse effects such as liver neoplasia (Myers *et al.*, 1998; Reichert *et al.*, 1998). As a result, histological analyses conducted on liver sections to detect early toxicopathic lesions, neoplasms, pre-tumors, and tumors are also recommended.

Bile sample analyses for PAH metabolites are conducted according to protocol and quality assurance and controls specified by National Marine Fisheries Science Center (NMFS, NOAA) in Seattle, WA. The facility maintains a rigorous QA/QC program for sample analysis. Laboratory studies have shown a strong correlation of PAH metabolites in bile with level of exposure (Britvic *et al.*, 1993; Collier *et al.*, 1991). This trend has also been observed in a number of field studies (Krahn *et al.*, 1992; Krahn *et al.*, 1993; McDonald *et al.*, 1995; Van der Oost, 1994).

Liver samples analyzed for DNA adducts and histological analyses are also conducted according to protocol and quality assurance and controls specified by National Marine Fisheries Science Center (NMFS, NOAA) in Seattle, WA. Because repair efficiency is low, DNA adduct formation in the liver is reflective of long-term PAH exposure (French *et al.*, 1996). Increased levels of DNA adducts are associated with increased prevalence of toxicopathological hepatic lesions in fish making it a sensitive indicator of toxic effects as well as exposure to PAHs

(Reichert et al., 1998).

Liver samples analyzed for ethoxyresorufin-*O*-deethlyase (EROD) activity are conducted by the Patuxent Analytical Control Facility of the U.S. Fish & Wildlife Service. EROD induction in fish is a well-established indicator of exposure to specific planar halogenated/polycyclic aromatic hydrocarbons and structurally related compounds. It is a highly sensitive indicator of contaminant uptake in fish providing evidence of receptor-mediated induction of cytochrome P450-dependent monooxygenases by xenobiotic chemicals (Whyte *et al.*, 2000). Mounting evidence indicates that the mechanism of CYP1A induction is closely related to, if not directly involved in, the detrimental effects seen in fish exposed to EROD-inducing contaminants (Whyte et al., 2000). Used in conjunction with other indicators of PAH exposure such as PAH metabolites in bile, detection in sediments, DNA adducts, and histological hepatic lesion analysis, EROD is a powerful technique for exposure assessment.

DATA ANALYSIS

PAH and mercury concentrations in fish tissue, biomarker results (EROD, PAH metabolites, DNA adducts) and hepatic lesions are compared for differences between samples collected at the SLRIDT Sites and reference sites. Mercury concentration uptake rates for fish are compared to determine differences between locations. Uptake rates are determined from the regression of concentration vs. length, age or weight of fish. Tumor prevalence greater than 2% is considered elevated (Hartig *et al.*, 1990). Baumann et al. (1996) reported that liver tumor prevalence exceeding 9% and skin tumor prevalence exceeding 20% were nearly always observed in chemically contaminated areas. Linear correlations are performed to determine associations between bioindicators, hepatic indicators or concentrations of PAHs. Additional analyses are conducted as deemed appropriate.

Statistical analyses are conducted using SigmaStat (SPSS, 1997). Determination of statistical significance is set at $p < 0.05$ for all tests. Statistical tests may include, but are not limited to: Shapiro-Wilks statistic to determine normality; analysis of variance (ANOVA), t-tests (assuming equal and unequal variances), and nonparametric Wilcoxon tests to determine differences between sites.

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