Final Study Plan
Fish Injury of the Lower Passaic River

Diamond Alkali Superfund Site Natural Resource Damage Assessment

DIAMOND ALKALI SUPERFUND SITE NATURAL RESOURCE FEDERAL TRUSTEES

U.S. Department of the Interior
U.S. Department of Commerce

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Executive Summary

The Lower Passaic River (LPR), located in Bergen County, Essex County, Hudson County, and Passaic County, New Jersey, is contaminated through the past and ongoing discharge of hazardous substances, including 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals. Diamond Alkali Superfund Site (DASS) Natural Resource Federal Trustees – the U.S. Department of the Interior, acting by and through the United States Fish and Wildlife Service, and the U.S. Department of Commerce, acting by and through the National Oceanic and Atmospheric Administration (collectively, the "Federal Trustees"), are conducting a natural resource damage assessment (NRDA) to assess and restore the natural resources injured and services lost due to those hazardous substances.

The LPR, which is part of the DASS, provides habitat for freshwater and estuarine fish species of multiple feeding guilds. Fish surveys conducted over the course of one year in 2009 and 2010 identified forty-five fish species throughout the lower 17.4 miles of the Passaic River (Windward 2019). The Federal Trustees aim to conduct laboratory and field studies in 2020/2021 to evaluate potential injuries to fish in the Passaic River due to hazardous substances, and also support the quantification of any such injury.

Pursuant to the DASS NRDA Plan, the Federal Trustees have developed this Study Plan to investigate fish injury in the Lower Passaic River. This Study Plan describes laboratory and field studies the Federal Trustees propose to undertake to meet the following objectives. The Federal Trustees seek to:

1. Assess potential injury to LPR fish embryos due only to maternal transfer.
2. Assess potential injury to uncontaminated fish embryos due only to exposure to LPR sediments (*i.e.*, no maternal transfer).
3. Assess potential injury to LPR fish embryos due to both maternal transfer and exposure to sediments from the LPR.
4. Assess potential injury to LPR adult fish resulting from long-term residence within the LPR.
5. Determine fish species presence and density in the LPR.

This study is aimed to assess potential injury to, and quantification of such injury to, lower trophic level fish species. An additional fish toxicity study will also be conducted in 2020/2021 to assess potential injury to higher trophic level fish species. The Federal Trustees may propose additional work to supplement this effort in the future. This plan was issued for a 30 calendar day public comment period, ending March 25, 2020. Public comments will be included in the Final Report of Assessment.

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1.0 Background

The Diamond Alkali Superfund Site (DASS) has released and continues to release hazardous substances, including 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals. The DASS Natural Resource Federal Trustees – the U.S. Department of the Interior and the U.S. Department of Commerce – are conducting a natural resource damage assessment (NRDA) to assess and restore the natural resources injured by those hazardous substances released at or from the DASS (DOI and NOAA 2020).

The Lower Passaic River, located in Bergen County, Essex County, Hudson County, and Passaic County, New Jersey, provides habitat for freshwater and estuarine fish species of multiple feeding guilds. Fish surveys conducted over the course of one year in 2009 and 2010 identified forty-five fish species throughout the lower 17.4 miles of the Passaic River (Windward 2019).

Fish are an integral part of the river ecosystem, and provide a number of important ecosystem services such as nutrient production, recycling, and transport. Fish are also an important source of prey for birds, mammals, and larger fish species. Fish may be exposed to hazardous substances through direct ingestion of contaminated water, sediment, and food items. Food items contaminated with hazardous substances derived from the LPR include other fish, amphibians, benthic invertebrates, adult insects that develop from aquatic larvae, and vegetation growing in the river. The dietary exposure of fish to these food items may result in the accumulation of hazardous substances in their tissues. This accumulation of hazardous substances may result in adverse physiological effects to adult fish, and potential adverse effects on reproductive success as hazardous substances are transferred to their eggs. In addition, eggs may be further exposed to hazardous substances once deposited onto either river sediment or vegetation proximate to river sediment.

The DASS NRDA Plan identified fish health as an area of biological injury investigation. As a means of evaluating DASS-related injury to fish species, the Federal Trustees propose to conduct laboratory studies in 2020/2021 to determine potential adverse effects on fish due to exposure to hazardous substances from the LPR. In addition, the Federal Trustees propose a field community survey to gather information on the presence and density of fish communities within the LPR, for potential use in injury assessment and restoration planning.

2.0 Introduction

Given the goals of the NRDA, the results of previous studies indicating the presence of a variety of fish species in the LPR, and data demonstrating high concentrations of hazardous substances in fish tissue sampled from the LPR (Windward 2011 and Windward 2019), the Federal Trustees have determined that it is appropriate to conduct further investigations to evaluate potential fish injury.

Pursuant to the DASS NRDA Plan, the Federal Trustees have developed this Study Plan for a fish injury assessment effort. This Study Plan describes:

1.) A multi-part laboratory effort to establish the nature and degree of survival and reproductive toxicity from LPR site conditions to both resident and reference fish, and
2.) A field fish community survey.

Laboratory toxicity testing will target the most sensitive developmental stages (embryonic and larval) and utilize several quantitative survival and reproductive endpoints to allow numerical calculation of adverse effects due to exposure to hazardous substances in the LPR. Additional laboratory testing will examine liver pathology in mature resident fish; this data is expected to provide numerical information on the frequency and severity of certain anticipated effects of chronic exposure to hazardous substances. Further, the field fish community survey is expected to provide information on fish community presence and density. All study elements will inform future injury quantification and restoration planning in the LPR.

3.0 Purpose and Objective

The purpose of this work is to inform the Federal Trustees' assessment of injury to fish, and to guide their future efforts to evaluate exposure pathways, as defined in regulations contained in Title 43 of the Code of Federal Regulations Part 11, Natural Resource Damage Assessment. This work will also be used to help determine the nature and scope of future studies.

The objectives of the laboratory and field studies the Federal Trustees propose to undertake pursuant to this Study Plan are:

1. Assess potential injury to LPR fish embryo-larval life stages due only to maternal transfer.
2. Assess potential injury to uncontaminated fish embryo-larval life stages due only to exposure to LPR sediments (*i.e.*, no maternal transfer).
3. Assess potential injury to LPR fish embryo-larval life stages due to both maternal transfer and exposure to sediments from the LPR.
4. Assess potential injury to LPR adult fish resulting from long-term residence within the LPR.
5. Determine fish species presence and density in the LPR.

4.0 Methods

Study Species

Four fish surveys were conducted between 2009 and 2010 as part of DASS remedial actions under U.S. Environmental Protection Agency oversight. Forty-five estuarine or freshwater fish species were identified throughout the LPRSA (Windward 2019, 2011). Of fish surveyed between 2009 and 2010, the majority of fish collected (87%) were classified as benthic

omnivores or invertivores/omnivores. The remaining fish caught were classified as planktivores (8%), invertivores/piscivores (3%), or piscivores (1%). Given that benthic omnivores comprise the majority of the LPR community structure, their ease of collection, and short reproductive cycles, the Federal Trustees propose mummichog (*Fundulus heteroclitus*), a benthic omnivore species, to be studied in the toxicity testing addressed in this plan. Mummichog comprised 24% of the total fish caught during the 2009 and 2010 surveys.

One advantage of using mummichog to conduct toxicity tests is that mummichog over 60mm long typically maintain a summer home range of 36-38m along one bank of tidal creeks (Abraham 1985). This small home range ensures that reproductive capabilities relate strongly to environmental conditions within the region of collection. A second advantage is that their short lifespan (reproductive at 2 years and lifespan of 4 years) allows for monitoring as frequently as every 3-5 years with no overlap of reproductive fish (Abraham 1985; Kneib and Stiven 1978).

In addition, mummichog are opportunistic and feed on almost any subtidal or intertidal benthic or water column organism (Weisberg and Lotrich 1982). Several studies conducted in varied habitats have demonstrated that the mummichog diet consists of detritus, algae, small crustaceans (*i.e.*, amphipods, tanaids, copepods, and ostracods), insects (adult and larvae), and polychaetes (Abraham 1985; Allen *et al.* 1994; James-Pirri *et al.* 2001; Kneib 1986; Currin *et al.* 2003). Mummichog often consume detritus incidentally while feeding on the water's surface or bottom substrate (Kneib 1986). The size of mummichog prey is limited by the size of the fish's mouth (Vince *et al.* 1976, as cited in Abraham 1985). Therefore, larger mummichog typically consume larger prey that are found at the water's surface or within the water column, whereas larval and juvenile mummichog, which are smaller and restricted to intertidal marsh or mudflat areas, have a diet that consists primarily of small benthic invertebrates (Kneib 1986).

4.1 Field Sample Collection

The Federal Trustees are planning to collect up to 24 sediment samples throughout the LPR during the month of July 2020. Once collected, ~250ml jars will be filled with homogenized sediment from each location and sent to analytical laboratories for chemical analyses. Sheepshead minnow (*Cyprinodon variegatus*) eggs will then be exposed to sediment of each sampling location. The results of both the chemical analyses and initial sheepshead minnow testing will be used to determine which sediment samples will best represent the range of hazardous substance concentrations for use in laboratory toxicity testing to be conducted in 2021.

Sediment

Sediment collection locations will be chosen to achieve an accurate and comprehensive representation of the types and concentrations of hazardous substances within the LPR. Sediment sampling locations were identified based on concentrations of hazardous substances in existing sediment grab and mummichog fish tissue data, along with mummichog collection locations and count data from the 2009 and 2010 fish community surveys, bathymetry data, and areas of the

river determined to be mummichog habitat. For each location, multiple sediment grabs will be taken using a randomly stratified sample design. Sediment grabs will be collected using a ponar grab sampler to target surficial material. The grab samples from each location will be combined to create a single composite for that location. A portion of each composite will be allocated to a pre-cleaned glass jar and sent for chemical analysis. Sediment will also be collected from the reference location, the Wye River in Queenstown, Maryland, for chemical analysis. Sediment used for toxicity testing will be placed on ice for transport to the aquatic toxicology laboratory.

Adjustments may be necessary during fish and sediment sample collection efforts to best accomplish the objectives of toxicity studies. The Federal Trustees and contracted field sampling team may make *in situ* decisions regarding sample numbers and locations based on unforeseen circumstances in the field. Any such decisions, and explanation for necessary changes, will be described in the report of assessment.

Fish

Collection locations will be chosen to achieve an accurate and comprehensive representation of adult mummichog that have been exposed to a range of types and concentrations of hazardous substances within the LPR. Locations will ultimately be dependent upon trapping an acceptable number of mummichog for toxicity tests within a proximate area. To constitute an acceptable number, enough gravid females will need to be collected for at least 2g egg composite sample for laboratory chemical analyses, as well as additional eggs for toxicity testing. Reference fish will be collected from a known population of uncontaminated mummichog located in the Wye River. Mummichog spawn during flood tides associated with new and full moons. Gravid female and male mummichog will be collected from the LPR and Wye River several days prior to a high tide cycle during the summer. Adult mummichog may be collected using multiple methods, including cast nets, minnow traps, gill nets, and seine netting. Following collection, male and gravid female mummichog will be transported alive for strip spawning in an aquatic toxicology laboratory at the University of Maryland Wye Research and Education Center in Queenstown, Maryland.

All samples collected will be labeled in a clear and precise way for proper identification in the field and for tracking in the laboratory. The samples will have pre-assigned, identifiable, and unique numbers. At a minimum, the sample labels will contain the following information: site location, date of collection, type of sample (i.e., sediment or fish), unique sample number, and analytical parameter(s) (which may be identified by using a key, such that “A” is equivalent to metals analysis, etc.).

4.2 Laboratory Chemical Analyses

The Federal Trustees will collect sediment and fish tissue composites from a variety of locations throughout the LPR, as well as one from the Wye River reference population, for laboratory chemical analyses. If an appropriate number of gravid female mummichog are collected at one

location to provide enough egg mass for a second composite sample, a field duplicate will also be sent for laboratory chemical analyses. Similarly, sediment composite samples will be sent for laboratory chemical analyses, along with at least one field duplicate of a composite sample. Pre-cleaned, sealed sampling containers will be provided by the analytical laboratory.

Fish tissue and sediment composite samples will be analyzed for dioxins/furans, PCBs, PAHs, and a full metals scan. Fish tissue composite samples will be homogenized in the analytical lab, and also analyzed for percent lipids and percent moisture. Sediment grab samples will be homogenized into one composite sample for each location. In addition to those listed above, sediment composite samples will also be analyzed for total organic carbon, percent solids, and grain size. Large non-sediment items (*e.g.*, rocks, woody debris, organisms) will be removed and noted. If sample homogenization is not practical in the field, samples will be brought to a local laboratory facility to be homogenized and aliquoted before shipment to the designated analytical laboratories for chemical analysis. Care will be taken to ensure samples are fully homogenized (*i.e.*, by visual inspection of sediment characteristics). Samples will be aliquoted into appropriate containers depending on the intended chemical analysis. Samples will be carefully sealed and labeled following collection and stored in coolers on ice prior to being transported to the laboratory. Fish and sediment samples will be shipped with appropriate hazardous shipping labels and permits for domestic and international transport to the analytical laboratories.

4.3 Laboratory Toxicity Studies

The Federal Trustees propose four laboratory toxicity studies to determine potential adverse effects of hazardous substances from the LPR on resident and reference fish. Three of the studies will focus on evaluating adverse reproductive effects: the Sediment Exposure (Reference Fish) Study, the Maternal Transfer Study, and the Maternal Transfer and Sediment Exposure Study. The following endpoints will be documented during these three studies, if possible:

- Percent fertilization;
- Time to hatch;
- Percent hatch;
- Survival at 5 days post-hatch in an environment with food provided;
- Weight at 5 days post-hatch;
- Number of days larvae survive in an environment without food provided;
- Developmental abnormalities in embryos and larval fish.

The fourth laboratory toxicity study will examine liver pathology in adult fish resident to the LPR, and if possible, the number of eggs per gravid female.

Sediment Exposure (Reference Fish) Study
This study will determine potential adverse effects due to exposure of LPR sediment to fish known to come from robust populations, with minimal history of contaminant exposure. Eggs will be stripped and fertilized from adult mummichog collected from the Wye River,

Queenstown, Maryland, a known reference location with low contaminant concentrations in water and sediment (Hartzell *et al.* 2017, Hartzell *et al.* 2018). These reference eggs will be placed in replicate beakers containing sediment collected from multiple locations throughout the LPR, as well as reference sediment collected from the Wye River as a negative control. A reference sediment will also be dosed with a reference chemical, such as 2,3,7,8-tetrachlorodibenzo-*para*-dioxin, as a positive control to determine expected adverse effects at a known concentration to mummichog and sheepshead minnow. Again, the locations chosen for sediment collections throughout the LPR will attempt to achieve an accurate and comprehensive representation of the range of concentrations and hazardous substances. Test procedures and toxicological endpoints will follow those described previously.

Eggs of the sheepshead minnow, a species with a 30+ year history of use in embryo-larval toxicity tests and of known sensitivity to a variety of contaminants, will also be exposed to sediment from multiple locations throughout the LPR as a negative and positive control. Inclusion of this test species will allow greater interpretation of survival and teratogenic effects and allow comparison of effects between the LPR and other historically impacted estuarine river systems. Tests methods will be based on EPA Method 1005.0 (EPA 2002) with modifications to incorporate sediment exposure within test vessels. Two well-established sources of sheepshead minnow embryos, Aquatic BioSystems (ABS) in Fort Collins, CO and Aquatic Research Organisms (ARO) in Hampton, NH, will be considered for supply of test organisms. Eggs will be shipped priority overnight and arrive at the laboratory at ≤24 hours post fertilization. As in the previous two studies, fertilized eggs will be counted and separated into groups of 15-20, then be placed into beakers containing sediment from specific LPR locations, and water with abiotic water quality parameters similar to the reference locations where adult mummichog were collected. A mesh screen will be placed over sediments to allow eggs to make sediment contact, but prevent burying of eggs. A water column of 2–3 cm above the sediment surface will be maintained during the entire test with water siphoned and replaced daily.

The sheepshead minnow tests will be run for approximately 10 days. This duration assumes approximately 5 days to hatch, and another 5 days to utilize the entire yolk sac and begin actively feeding. At this time, surviving larvae will be weighed to generate a growth endpoint. Hatching time course will be determined by counting the number of viable larvae hatched every 12 hours beginning at 96 hours post-fertilization. Observations will continue until 48 hours after 100% of the larvae have hatched in the control treatment.

Maternal Transfer Study

The purpose of this study is to evaluate potential adverse effects, due to maternal transfer only, in embryo-larval life stages of mummichog collected from the LPR. Once in the laboratory, eggs from multiple gravid female mummichog from the same collection location will be stripped, combined, and fertilized following the methods of Ownby *et al.* (2002). Prior to fertilization a 2-

5g sub-sample of eggs from each location will be set aside for chemical analyses. Sperm from multiple males will be composited and used to fertilize eggs from the same collection location. Eggs will be counted, separated into groups of 15-20, then placed into replicate beakers containing water with abiotic water quality parameters similar to the individual location where adult mummichog were collected. Water will be replaced once per day for the duration of the study. These replicate beakers will be used for the purposes of determining hatching success, as well as survival and growth to 5-days post hatch. Mummichog eggs from the reference location, with no known maternal exposure to dioxin-like compounds or genetic tolerance to dioxin-like compounds, will also be placed in separate beakers to be used as a control.

Test methods will follow those described in EPA Method 1005.0 (Sheepshead Minnow, *Cyprinodon variegatus* Embryo-Larval Survival and Teratogenicity Test: Chronic Toxicity) (EPA 2002) with modifications, as necessary, to accommodate the longer mummichog embryonic period. Mummichog embryonic development coincides with new and full moon flood-tide cycles so requires approximately 14 days from fertilization to hatch. Therefore, mummichog hatch and survival tests will be run for a 14-day embryonic period, followed by a 5-day post-hatch period. At the conclusion of the 5-day post-hatch period, surviving larvae will be fixed (70% ethanol) for examination of developmental abnormalities (*e.g.*, corneal, cranial, spinal, and fin development) before being dried and weighed to generate a growth endpoint.

Separate replicate beakers of fertilized embryos will be sacrificed for examination at 5 days post fertilization to identify developmental abnormalities following the methods of Ownby *et al.* (2002). Cardiovascular deformities will be quantified using a modified cardiovascular index (CVI) developed by Weis and Weis (1984), that ranks defects in heart structure and function in live embryos. Subsequently embryos will be fixed (70% ethanol) for examination of other developmental abnormalities.

Maternal Transfer and Sediment Exposure Study

Due to the environment of a highly industrial, urbanized, and tidal river system, the logistical risks of conducting a field exposure study in the LPR outweigh the likelihood of capturing meaningful results. Given this, the Federal Trustees propose a laboratory study using sediment collected from the river to simulate similar exposure conditions to fish embryo-larval life stages as a field study. The purpose of this study is to evaluate potential adverse effects in mummichog fish embryos due to both maternal transfer and exposure to sediments collected from multiple locations throughout the LPR. Therefore, sediment embryo-larval life stage exposures will be performed coincident with, and employ subsets of, the mummichog embryos sourced from the various locations for the maternal transfer study.

As before, eggs from multiple gravid female mummichog from the same collection location will be stripped, combined and fertilized following the methods of Ownby *et al.* (2002). Sperm from multiple males will be composited and used to fertilize eggs from the same collection location. Eggs will be counted, randomly separated into groups of 15-20, then placed into replicate

beakers containing sediment from the same location. Beakers will also contain water with water quality parameters similar to individual locations where adult mummichog were collected. Eggs from each LPR location will also be placed into beakers containing reference sediment collected from the Wye River.

Test methods will be based on EPA Method 1005.0 (EPA 2002). Modifications will be made to the glass tubes to accommodate for differences in mummichog embryonic developmental requirements, as well as to incorporate sediment exposure within test vessels. A mesh screen will be placed over sediments to allow eggs to make sediment contact, but prevent burying of eggs. Sufficient water will be maintained above the sediment surface to keep mummichog eggs moist but not entirely submerged. After 14 days post-fertilization, water level will be increased to 2-3 cm above the sediment surface to simulate a flood tide. As described previously, the mummichog embryonic period is 14 days to coincide with new and full moon flood-tide cycles, so mummichog tests will be run for a 14-day embryonic period, followed by a 5-day post-hatch period. At the conclusion of the 5-day post-hatch period, surviving larvae will be fixed (70% ethanol) for examination of developmental abnormalities (*e.g.*, corneal, cranial, spinal, and fin development) before being dried and weighed to generate a growth endpoint.

Beakers of fertilized embryos will be examined at 5 days post-fertilization to identify developmental abnormalities following the methods of Ownby *et al.* (2002). Cardiovascular deformities will be quantified using a modified cardiovascular index (CVI) developed by Weis and Weis (1984), that ranks defects in heart structure and function in live embryos. Subsequently embryos will be fixed (70% ethanol) for examination of other developmental abnormalities.

Adult Fish Study

This study will quantify the frequency and severity of liver lesions in populations of fish resident within the LPR. Mummichog are epibenthic forage fish consuming detritus as well as algae and aquatic plants and a variety of animal taxa including crustaceans (copepods, ostracods, amphipods, tanaids), insects (adult and larval), mollusks (snails, softshell clams) and more (Baker-Dittus 1978). They have substantial sediment contact during foraging and are also reported to burrow into sediment during winter (Chidester 1920). In this way they receive dietary, respiratory and dermal routes of contaminant exposure. These characteristics, coupled with their very small home range (Abraham 1985), make mummichog a useful indicator of regional environmental quality. The liver is the primary target organ of chronic toxicity due to its significant roles in blood filtration and contaminant metabolism (Di Giulio and Hinton 2008). Therefore, lesions within the liver are a useful indicator of long-term contaminant exposure (Vogelbein *et al.* 1990).

Mature mummichog (total length ≥75 mm) will be field collected from the LPR and transported live to the toxicology lab for the purpose of investigating liver pathology. Upon return to the laboratory, fish will be euthanized, examined grossly, sexed, measured (standard and total

lengths), and weighed. If possible, the number of eggs will be counted for each gravid female. Livers will be removed and preserved (10% neutral buffered formalin) before being processed by routine methods for paraffin histology (Luna 1968). Liver pathology will also be performed on fish collected from the Wye River to determine the prevalence of lesions in reference areas. Handling of fish during collection, transport, holding, euthanasia, and tissue dissection will be in accordance with Institutional Animal Care and Use Committee protocols. Histological processing and evaluation of liver lesions will be performed at the Virginia Institute of Marine Science, Gloucester Point Virginia.

4.4 Field Community Survey

The Federal Trustees intend to conduct surveys to document current fish and crab communities in the LPR, and the lower Raritan River (LRR) as an estuarine reference river for comparison. Because salinity is one of the dominant environmental factors structuring estuarine fish assemblages (Able and Fahay 2010), survey areas in the LPR will be paired with locations in the LRR of similar salinity. Surveys are currently planned for fall 2020, spring 2021, summer 2021, and fall 2021. The timing of these surveys aim to document anadromous species that have moved from the ocean into the river in the spring, as well as juvenile fish growth. A variety of sampling methods may be used in various habitat types to target different fish species, including minnow traps, trotlines, cast nets, gillnets, beach seine nets, and trawl nets. Multiple survey methods may be used throughout the river depending on water depth, riprap/vegetation, and anticipated species that are likely to be present.

Survey Methods

The Federal Trustees will collect juvenile and adult fishes, as well as crabs, to measure species composition, size, abundance, and environmental and habitat variables. In addition, site-specific environmental data will be collected concurrent with survey methods. Water quality data (*e.g.*, salinity, temperature, pH, dissolved oxygen) and water depth will be collected at each site visit. Classified bottom type will be extracted from available GIS shape files. Other bottom type characteristics may be noted based on expert review of field observation and side scan sonar images collected at each sample site prior to fish survey sampling. Sampling will be repeated seasonally (*e.g.* spring, summer, and fall) resulting in three sampling rotations for both the LPR and the LRR.

Depending on field conditions (water depth, debris along river bottom, *etc.*), nearshore habitat, and species likely to be present at various survey locations throughout the LPR and LRR, fish and crabs will be sampled using one, or a combination of equipment, including otter trawls, minnow traps, trot-lines, and gillnets. For each sampling method, all fishes captured will be identified and counted. Up to twenty fish of each species will be randomly measured to the nearest mm total length (TL) or fork length (FL) (depending on appropriate length measurement for each species). For samples containing more than 20 individuals of a particular species, the

length frequency distribution based on 20 specimens will be assigned to the total catch for that species in that capture event as a proportion.

Data Analysis

During surveys, the number of fish and crabs per species will be recorded, along with a metric of fishing effort (*i.e.*, area swept for the trawl and trap, hook, or meter hours for the minnow trap, trot-line, and gillnet, respectively) for each gear type and location to calculate catch-per-unit-effort (CPUE). CPUE will be calculated for both the LPR and LRR. CPUE is calculated as the ratio of the number of specimens caught at a given location divided by the amount of fishing effort (defined above) required to catch those specimens at that location.

5.0 Quality Assurance/Quality Control

5.1 Laboratory Analyses

All samples will be shipped to an analytical laboratory for chemical analysis. Samples will be homogenized and extracted for chemical analysis. It is important ensure that data of sufficient and known quality are generated through the implementation of this study. The contracted analytical laboratory will submit written Standard Operating Procedures (SOPs) detailing their respective methods and Quality Control (QC) and Quality Assurance (QA) procedures. Accepted EPA and American Society for Testing and Materials (ASTM) methods will be used to determine the concentrations of dioxins and furans, PCBs, PAHs, and metals in field-collected (Reference and Target Areas) sediment and fish tissue. Modifications to the approved SOPs will be documented in the final study report describing the field collection activities and laboratory results.

Laboratory QC samples will be used to assess the effects of homogenization procedures and to evaluate the potential for laboratory-derived contamination, laboratory performance, and sample matrix effects. Quality control samples may include: method blanks, laboratory duplicate samples, certified reference material (CRM) and egg reference (control) material analyses. Surrogates or labeled analog compounds will be added to each sample to further assess the effects of sample matrix on accuracy. The analytes of concern for this study are persistent compounds, which have been found to remain stable in tissue after several years of storage. Therefore, no maximum holding time criterion will be established for data validation. Samples will be kept frozen while in archive.

Sample results and related QC data will be received in an electronic data deliverable (EDD) format. The analytical data packages submitted by the laboratory will be reviewed to determine whether the Data Quality Objectives are met, with additional details to be included in analytical data validation reports.

The Federal Trustees intend to develop procedures and schedules for sharing data, split samples, and results of analyses, when requested, with any identified potentially responsible party. Information on any such decisions and procedures will be shared with the public.

5.2 Sediment Collection and Field Surveys

All field-collected information is recorded in GPS units, bound field notebooks, and field forms, which shall be signed and dated. To ensure quality of data, two forms of QA will occur:

- Manual transcription verification which will ensure that all information from the field data sheets was accurately recorded in the electronic file.
    - USFWS staff will manually check for accuracy the first five data sheets transcribed by each field surveyor. If errors are identified, they will be fixed and the field surveyor will be notified. If no errors are identified, USFWS will manually check up to an additional five percent of data sheets entered by each field surveyor.
- Automated screening of entries to identify entries that fall outside the range of likely possibilities, including:
    - Identifying numeric entries at the extreme ends of potential ranges, possibly indicating the entry was in the wrong units.
    - Cross referencing species recorded against possible species known to frequent the area.
    - Reviewing all species names to ensure consistent spelling.

In addition, the Federal Trustees will conduct a qualitative evaluation of the operational details at a date to be determined, without notifying the contracted field surveyors in advance. This evaluation will include, but not be limited to, a review the field equipment being used, the consistency with which the methods are followed, and the storage of paper data sheets. In addition, the Principal Investigator of the project will accompany field staff on a regular basis to ensure they are accurately identifying species.

To evaluate the outcome of this study, the PI will compare the observed densities to those reported for the same species elsewhere. Such a comparison will allow for the identification of estimates greater than or less than a species reported density in the literature.

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