Mississippi Canyon 252

ANALYTICAL ADDENDUM: ASSESSMENT PLAN TO DETERMINE POTENTIAL EXPOSURE AND INJURIES OF NESTING AND HATCHLING KEMP'S RIDLEY AND LOGGERHEAD SEA TURTLES AND THEIR NESTS:

SAMPLE ANALYSIS PLAN FOR YEARS 2010 /2011

Approval of this Kemp's ridley and loggerhead analytical addendum is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

This plan will be implemented consistent with existing trustee regulations and policies. All applicable state and federal permits must be obtained prior to conducting work.

The trustees have developed a preliminary conceptual model of the DWH release, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the trustees' decision to pursue the studies outlined in the work plan.

Kevin D. Reynolds — Department of the Interior Trustee Representative — Date: 8/24/2012

Louisiana Trustee Representative — Date: 9/13/12

Texas Trustee Representative — Date: 8/30/2012

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ANALYTICAL ADDENDUM: ASSESSMENT PLAN TO DETERMINE POTENTIAL EXPOSURE AND INJURIES OF NESTING AND HATCHLING KEMP'S RIDLEY AND LOGGERHEAD SEA TURTLES AND THEIR NESTS: SAMPLE ANALYSIS PLAN FOR YEARS 2010 /2011

Background

Field investigations of the impacts of the *Deepwater Horizon*/Mississippi Canyon 252 Oil Spill and subsequent response efforts (the "MC252 Oil Spill") on endangered Kemp's ridley and threatened loggerhead sea turtles, performed in 2010 and currently underway for 2011, investigate the occurrence of Mississippi Canyon 252 (MC 252) oil and dispersant (hereafter referred to as "MC 252 oil") exposure and resulting effects in order to quantify injury for the purposes of a Natural Resource Damage Assessment (NRDA). As a part of each year's study, collected samples will undergo analytical procedures to quantify levels of exposure and subsequent physiological, developmental and toxicological effects. The purpose of this Analytical Addendum is to document the types of analyses to be conducted, and approximate the number of each sample type and analyses to be performed. Analyses fall into two classes, chemical and biological:

Chemical analyses include screens for petroleum components (including polycyclic aromatic hydrocarbons, or PAHs), PAH metabolites, chemical dispersants used to disperse the petroleum contaminants, and halogenated aromatic hydrocarbons (HAHs).

Biological analyses include analyses used to characterize the sex of hatchlings; familial relationships between adult sea turtles and their nests; the foraging patterns, clinical chemistry and hematology of nesting females; and the biochemical response of turtles to MC252 Oil Spill-associated contaminant exposure.

Analyses will be performed on a variety of tissues and materials from:

Adult nesting female turtles: Carapace and skin wipes, blood and carapace and skin biopsies

Hatchling survivors: Chorioallantoic membranes (CAMs)

Mortalities: Hatchling and late term embryo: CAMs, Residual yolk, liver, bile, muscle, gonads

Cracked or otherwise impaired, unincubated and unhatched eggs: Pooled/composited egg contents of each type for each nest, separately

Nests: Nest-associated sand

As most adult female nesting activities were complete by the time sampling programs were initiated, the majority of samples for 2010 were from unhatched eggs and hatchlings. In addition

to samples similar to those collected in 2010, 2011 samples will include more tissues from nesting female turtles and sand from Kemp's ridley nests.

Table 1 (for Kemp's ridleys) and Table 2 (for loggerheads) describe each of the different tissues/matrices, the analytical procedures to be performed, and the numbers of samples that were collected in 2010 and anticipated in 2011.

Role of Sample Analyses in the NRDA Process.

Each analysis plays a role in assessing exposure to petroleum contamination and/or the effects of such exposures resulting from the MC252 Oil Spill that may be indicative of injury. Some analyses provide both exposure and effects information. The following describes the chemical, biological and satellite transmitter analyses that will be implemented, and the samples (and their sources) upon which these analyses will be performed.

Chemical Analyses

Preliminary methods development will occur prior to analyses and in coordination with the Deepwater Horizon (DWH) NRDA Chemistry Technical Working Group (TWG) and NRDA-contracted analytical chemistry laboratories. The results of analyses from this addendum will be integrated with data from oiled, rehabilitated or dead turtles collected as part of spill-associated response efforts.

For many tissue types, relatively small numbers of specimens exist and can be managed easily. However, large sample numbers of other tissues (e.g., eggs) will need to be prioritized for analysis. The actual level of exposure of each turtle will not be known, but positive findings from other analysis and field observations will be considered in selecting the first samples to be analyzed. By prioritizing samples from oiled turtles, oiled nests, oiled eggs, tagged turtles that have been foraging in areas impacted by the MC252 Oil Spill, or other such examples, the chances of finding target analytes may be increased. The most likely impacted samples will be analyzed initially since detections early in the process will provide assurances of the efficacy of the analytic method developed. These initial analyses will determine the processing of samples going forward.

Petroleum and PAH Analyses. Chemical analyses of the full spectrum of petroleum components (total extractable hydrocarbons/saturated hydrocarbon compounds (TEH/SHC as indicated in Table 1.1b of the DWH Analytical Quality Assurance Plan)), and more focal PAH/petroleum biomarker compounds, provide both qualitative and quantitative profiles of oil exposure. Samples originating from internal matrices and externally collected samples provide complementary information. Samples originating from internal matrices, such as blood, eggs and CAMs, are generally limited to analysis of PAH compounds as these are the compounds that are absorbed through biological membranes and exhibit toxic effects (Short and Springman 2007, Fournier et al. 2010). Externally collected samples (carapace/skin wipes, sand) can provide broader component profiles including TEH/SHC and more complex compounds such as terpanes, hopanes, steranes and other 'petroleum biomarkers' in addition to PAHs (Wang et al. 2007).

- *Sample numbers* (X/Y = 2010/ anticipated 2011 for each species noted; see Table 1 for specific tissue/year analysis breakdown for Kemp's ridleys and Table 2 for loggerheads):

Kemp's ridley: Petroleum components/PAH/Petroleum biomarkers – 1/40;
PAH/Petroleum biomarkers - 118/160
Loggerhead: Petroleum components/PAH/Petroleum biomarkers - 20/70;
PAH/Petroleum biomarkers - 364/70

- *Analysis method:* Methods consistent with the DWH AQAP – primarily gas chromatography (GC)/flame ionization detection for TEH/SHC and GC/mass spectrometry for PAHs and petroleum biomarkers

- *Analytical laboratory:* DWH NRDA contract laboratory to be determined.

PAH metabolites. PAH metabolism in vertebrates, both activation and degradation, is rapid, with little remaining parent material lasting more than 24 hours following exposure in many species. Metabolically hydroxylated (as sulfate and glucuronide conjugates) PAHs are sometimes all that remain from a PAH exposure (Short and Springman 2007), and are assessed both qualitatively and quantitatively in a manner similar to the petroleum screen (Beyer et al. 2010). These analyses expand the range of compounds found in an organism, as the metabolites can be combined with the parent compounds to obtain a better exposure profile. The waste products of PAH metabolism, these compounds can be measured in the blood of the nesting female and egg yolks to assess maternal contributions. They will be measured in bile from gall bladders of embryo and hatchling mortalities and in the contents of CAMs from both hatchling survivors and hatchling/embryo mortalities to assess embryonic exposure.

- *Sample numbers:* Kemp's ridley: PAH Metabolites – 233/220
Loggerhead: PAH Metabolites – 359/50

- *Analysis method:* High performance liquid chromatography (HPLC) with direct fluorescence detection (Gale et al. In review)
- *Analytical laboratory:* Environmental Chemistry Branch, USGS Columbia Environmental Research Center, Columbia, Missouri.

Dispersants. Chemical dispersants were used during the MC252 Oil Spill to break-up oil and speed its dissipation and degradation. These compounds may have become associated with residual oil where it continues to exist in environmental depots and, thus, may be available for turtle exposure. Chemical analysis of dispersants can be done on many of their constituents, though the focus to date has been on a relatively persistent anionic surfactant, DOSS (dioctylsulfosuccinate). Planning for analyses of these compounds awaits further development of analytical methods by the Trustees.
- *Sample numbers:* TBD
- *Analysis method:* DWH NRDA contract laboratory methods – analysis of the marker compound, DOSS
- *Analytical laboratory:* DWH NRDA contract laboratory to be determined.

HAH (halogenated aromatic hydrocarbon) Compounds. Certain biochemical endpoints analyzed as indicators of PAH exposure, such as CYP1A activity or immunohistochemistry, are known to also be responsive to HAHs when concentrations are elevated in study organisms. HAHs could be present in the northern Gulf environment from sources other than the MC 252 explosion and oil spill, leading to uncertainty as to the cause of a measured biochemical response. Should a biochemical response be documented in tests implemented under this Analytical Addendum, it may be useful to determine if HAH concentrations are sufficiently elevated to cause the response. Analysis for HAH concentrations in tissues from adult turtles, eggs or embryos demonstrating biochemical responses considered characteristic of PAH exposure could eliminate the possibility that such responses are caused by HAHs (Trust et al. 2000) if no HAHs are found. Up to 20 samples may be tested for HAH concentrations, based on PAH exposure and biochemical response data that suggest the analyses could provide clarity or reduce uncertainty in exposure or injury assessments.

- *Sample numbers:* 10/10 each for Kemp's ridley and Loggerhead. A total of 40 analyses will be performed if the trustees determine that such analyses would be helpful for clarifying findings.
- *Analysis method:* Extraction and cleanup followed by analysis using: Gas chromatography (GC)/high-resolution mass spectrometry for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and GC with electron capture detection with dual column confirmation for PCBs (Echols et al. 2004, Gale 2007, Augspurger et al. 2008).
- *Analytical laboratory:* Environmental Chemistry Branch, USGS Columbia Environmental Research Center, Columbia, Missouri.

Biological Analyses

Hatchling Gonad Histopathology. As sea turtles exhibit temperature-dependent sex determination, simple genetic analysis cannot provide identification of hatchling sex (Wibbels 2003). Sex identification is accomplished through histopathological examination of gonads in late-stage embryo mortalities collected from across incubated nests. Temperatures maintained at the Padre Island National Seashore incubation facility result in production of approximately 75% female embryos and hatchlings. Laboratory studies of oil in turtle nestlings suggest the potential for skewing of sex ratios toward males (Rowe et al. 2009). Sea turtle populations are limited by the number of female nesting adults, therefore, reductions in the number of such females could have profound effects on the population dynamics of this species.
- *Sample numbers:* Kemp's ridley: 722/250 (note - 2010 value represents samples from 2008, 2009 and 2010 in order to maintain continuity of findings from turtles hatching from the Kemp's ridley incubation program)
- *Analysis method:* Formalin-fixed kidney/gonad/adrenal complex will be embedded in paraffin, sectioned at 8 um, mounted onto slides and stained with hematoxylin and eosin. Hatchling sex is based on anatomy of the gonads: ovaries exhibit a distinct cortex and an unorganized medulla, whereas testes lack a cortex and have an organized medullary region with developing seminiferous tubules (Wibbels 2003, Yntema and Mrosovsky 1980).
- *Analytical laboratory:* DWH NRDA contract laboratory to be determined.

Maternal and Hatchling Tissue DNA. Nesting Kemp's ridley sea turtles lay, on the average, between two and three clutches per season. When observed laying their nests on the beach, the contents and condition of the nests can be tied to a specific adult female turtle. However, approximately half of the Kemp's ridley nests are discovered after the female has returned to the ocean. For these nests, tissue DNA analysis is needed to attribute nests to specific nesting females.
- *Sample numbers:* Kemp's ridley: 370/370
- *Analysis method:* Genotypes developed from skin biopsies or blood taken from adult nesting females will be compared to those from embryo and hatchling mortality tissues collected from each nest using methods documented in Frey et al. 2008 and 2010. Specifically, DNA will be extracted from tissue (silica-based method) or blood (lysis method), and genotypes assembled with 11 loci. Cervus v3.0 will be used to match nesting females to nests.
- *Analytical laboratory:* Marine Turtle Research Program, NOAA Southwest Fisheries Science Center, La Jolla, California.

Isotopic Ratios in Maternal Scute Biopsies. The nature of isotopic profiles in the many species that have been tested depends, to a large part, on the trophic level of dietary items consumed, with distinctive increases in the stable nitrogen and carbon isotopes as animals shift foraging strategies from herbivorous to omnivorous to carnivorous diets (Hobson et al. 1994). Stable isotopes of carbon and nitrogen from the diet of Kemp's ridley sea turtles accumulate in the keratinous scute tissues that form over the turtle's bony carapace. Sampling of scute biopsies from Kemp's ridley sea turtles is performed to determine if the animals have made a shift in their dietary base of primarily crab species to increased proportions of lower trophic species such as jellies or salps (Reich et al. 2007). Such a shift would be suggestive of depletion of higher trophic level prey items in their diet. Scute biopsies from nesting females will be analyzed for stable carbon and nitrogen isotopes as a function of the age of the scute material that is laid down, as progressively deeper layers of scute tissues represent more recent foraging behavior, while surficial layers contain the oldest retained foraging history. A progression of isotopic profiles is developed to allow characterization of foraging patterns through time from before the MC252 Oil Spill to the present.
- *Sample numbers:* Kemp's ridley: 15/45
- *Analysis method:* A scute sample is collected with a 6 mm biopsy punch at the time of egg laying. The sample is micro-sampled in successive layers (50 um) to provide a chronological sequence. Each layer is combusted separately in an elemental analyzer interfaced to an isotope ratio mass spectrometer (Reich et al. 2007).
- *Analytical laboratory:* Sea Turtle and Fisheries Ecology Research Lab, Department of Marine Biology, Texas A&M University, Galveston, Texas.

Clinical Chemistry and Hematology of Maternal Blood Samples. Sea turtle blood samples can provide health information through clinical chemistry and hematology assessments. A standard clinical chemistry analysis of a plasma sample provides data on electrolytes, minerals, enzymes and other important biomolecules. Results from clinical chemistry panels yield insights on liver, kidney and cardiac function as well as overall homeostatic condition (Lutcavage et al. 1995). Hematological evaluations are composed of a blood smear created from heparinized blood sample and the measurement of packed cell volume (or hematocrit).
- *Sample numbers:* Kemp's ridley: 0/20

Loggerhead: 0/20

- *Analysis method:* Blood smears (3 per sample) will be prepared on slides and air dried prior to storage and shipment. Heparinized blood will be pulled into a hematocrit capillary tube, spun and read for packed cell volume. Heparinized whole blood will be centrifuged and plasma will be removed and frozen prior to submission for clinical chemistries. Standard veterinary clinical chemistry methods will be performed with an autoanalyzer capable of running multiple endpoints on each sample.
- *Analytical laboratory:* DWH NRDA contract Laboratory to be determined.

Cytochrome P450 Enzyme and Immunohistochemistry Activities. Activity of cytochrome P450 1A (CYP1A) can reflect internal PAH exposure through an induction process that leads to increased activity of this metabolic enzyme. In studies with birds, embryonic CYP1A activity has correlated positively with embryo toxicity (Grandberg et al. 2003). As the only tissues available from live sea turtle hatchlings are the residual CAMs following hatchling emergence, CYP1A provides a non-lethal measure of exposure and potential toxicity for this endangered species. For the purposes of this study, where the CAM is present in the nest for up to 24 hrs post hatch, CYP1A is measured using both activity and immunohistochemistry methods. As a positive response of this marker is not exclusively attributable to PAH exposure, samples may also be analyzed for halogenated aromatic hydrocarbon to determine whether PCB, dioxins or other halogenated inducers may be a contributing cause of the response (Trust et al. 2000).

- *Sample numbers:* Kemp's ridley: 130/240
Loggerhead: 26/0 (source: Kennedy Space Center translocated nests)
- *Analysis method:* Activity is measured in tissue preparations (from frozen tissue samples) as the dealkylation rate of a series of resorufin ethers (methoxy-, ethoxy-, pentoxy- and benzoyloxy-resorufin) to resorufin in a fluorometric assay (Burke et al. 1985, Cole et al. 2010) and expressed as a function of protein concentration. Immunohistochemistry utilizes an antibody to the CYP1A enzyme applied to a histological section of the tissue (formalin-fixed and stored in ethanol) and visualized with a peroxidase/anti-peroxidase detection system (Prophet et al. 1994, Godard-Codding et al. 2011).
- *Analytical laboratory:* Genomic and Cellular Ecotoxicology Lab, The Institute of Environmental and Human Health, Texas Tech University, Lubbock, Texas.

Kemp's Ridley Tissue Culture Toxicity Assessments. Because of their endangered status, methods to detect and evaluate relevance of contaminant impacts in Kemp's ridley sea turtles will be performed in cell and/or organotypic cultures of sea turtle tissue biopsies exposed to PAHs relevant to the DWH impacted marine environment (Godard et al. 2004, Cole et al. 2010). Concurrent with CYP1A-type assessments used in field-collected samples, other endpoints relative to tissue toxicity and damage, including oxidative stress, cell leakage and cell death assays, will be measured. Responses observed in these cultures may inform efforts to assess potential effects in nestling and embryonic sea turtles due to exposures to DWH-related compounds.
- *Analysis method:* Tissues used in these studies are isolated from stranded or rescued Kemp's ridley sea turtles that die or are euthanized for humane reasons. Tissues will be express-shipped to the laboratory where they will be processed and maintained as cells or isolated tissue slices, whichever prove most useful in pilot studies (Cole et al. 2010). These cultured tissues will then

be exposed to PAH profiles at concentrations relevant to those associated with MC252 oil exposed turtles. Choice of PAHs used to challenge cultures will be based on DWH-associated tissue PAH analysis data from rescued and nesting sea turtle studies, and further informed by Trustee studies of water accommodated fraction (WAF) and chemically-enhanced WAF (CEWAF) of DWH-associated petroleum. Response endpoints will include CYP1A response, oxidative stress, cell leakage and cytotoxicity. CYP responses will be compared to CYP responses in field-collected samples.
- *Analytical laboratory:* Genomic and Cellular Ecotoxicology Lab, The Institute of Environmental and Human Health, Texas Tech University, Lubbock, Texas.

Satellite Transmitter Data Analyses

Number of Nesting Females Potentially Exposed and Affected and the Extent of such Exposure. A model will be developed by overlapping sea turtle satellite transmitter data with maps of the date and location of the MC 252 oil in the water. Hindcasting will be performed using satellite-tracking data for nesting loggerhead females tagged as part of the 2010 and 2011 loggerhead NRDA work plans, along with data products such as the Integrated Oil Spill Impact Model System (SIMAP). A similar approach will be used for data from the Kemp's ridley females tagged as part of the 2010 and 2011 Kemp's ridley NRDA work plans. Using a GIS approach, SIMAP, or other exposure indicators, turtle data will be overlain to match temporal and spatial resolution of oil and turtle track layers. In addition, data available from other relevant satellite tracking studies and DWH spill distribution assessments may also be utilized in this model.

A state-space modeling (SSM) approach will be used to analyze the movements of both loggerhead and Kemp's ridley females in the Gulf of Mexico. Specifically, to better understand movement and foraging behavior of these two study species in oiled areas, we will fit a modified version of the Jonsen et al. (2006) two-state behavioral switching model and/or a modified version of the Breed et al. (2009) or Hart et al. (submitted) SSMs to all available loggerhead tracks (Ns are 16 for 2010 NRDA and 20for 2011) and Kemp's ridley tracks (Ns are 9 for 2010 NRDA and 13 for 2011) and potentially other loggerhead and Kemp's ridley tracks publicly available on www.seaturtle.org or contributed by other collaborative investigators.

All modeling approaches will be performed in an iterative fashion to allow reasonable additional inputs and interpretation. Model outputs will be developed with the intention that they can be integrated with other DWH modeling efforts underway.
- *Analysis method:* See above for details
- *Analytical laboratory:* USGS Southeast Ecological Science Center, Davie, Florida.

Sample and Data Handling

MC 252 NRDA chain-of-custody procedures will be observed at all times for all samples. All samples will be transferred with appropriate chain of custody forms and all samples that will undergo chemical or other analyses will be shipped to the appropriate laboratories for processing and analysis. Digital photographs of embryo mortalities will be collected on the camera-linked computer hard drive in write-protected read-only files, and backed up on an external hard drive

under a similarly protected file status until downloaded to the DOI data repository for the DWH NRDA.

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, including any remains of samples and including remains of extracts created during or remaining after analytical testing, must be preserved and disposed of in accordance with the preservation and disposal requirements set forth in Pretrial Orders ("PTOs") # 1, # 30, #35, # 37, #39 and #43 and any other applicable Court Orders governing tangible items that are or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Destructive analytical testing of oil, dispersant or sediment samples may only be conducted in accordance with PTO # 37, paragraph 11, and PTO # 39, paragraph 11. Circumstances and procedures governing preservation and disposal of sample materials by the trustees must be set forth in a written protocol that is approved by the state or federal agency whose employees or contractors are in possession or control of such materials and must comply with the provisions of PTOs # 1, # 30, # 35, 37, #39 and #43.

All laboratory data will be managed and stored in accordance with written SOPs. The appropriate training on particular equipment or in the conduct of specific laboratory studies for all personnel involved with the project shall be documented and those records kept on file for the duration of this project.

Copies of analytical and non-analytical data will be provided to the Louisiana Oil Spill Coordinator's Office (LOSCO) within a reasonable timeframe once data collection, QA analyses and data entry procedures are complete but no later than 6 months following its generation. All samples collected pursuant to this Addendum will be submitted to laboratories that are operated in a manner that is consistent with the guidelines of the Analytical Quality Assurance Plan for the Mississippi Canyon (Deepwater Horizon) Natural Resource Damage Assessment (version 3.0).

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT) and the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana. The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' DMT. Any preliminary data distributed to the DMT shall also be distributed to LOSCO. Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Analytical Quality Assurance Plan, after which time the validated/QA/QC'd data shall be made available simultaneously to all trustees. Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Analytical Quality Assurance Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT or LOSCO prior to validation/QA/QC absent a showing of critical operational need.

Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees.

Budget

The total laboratory costs for this 2010-2011 Analytical Addendum is $1,857,782. The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher.

Principle Investigator

Michael Hooper (573-441-2985, mhooper@usgs.com), U.S. Geological Survey, Columbia Environmental Research Center

Other Investigators

Donna Shaver (361-949-8173, ext. 226, Donna_Shaver@nps.gov), National Park Service
Role: Oversight of all field activities, nest incubation and sample collection, and transmitter implementation, Padre Island National Seashore.

Céline Godard-Codding, (celine.godard-codding@tiehh.ttu.edu), Texas Tech University
Role: Oversight and direction of all laboratory sample collection documentation and stabilization activities and CYP and tissue culture studies.

Kimberly Reich (reichk@tamug.edu), Andre Landry (landrya@tamug.edu), Texas A&M University at Galveston
Role: Oversight and direction of upper Texas coast Kemp's ridley field monitoring, sample collection and transmitter implementation. Leads scute isotopic analysis activities.

Kristen Hart (954-236-1067; Kristen_Hart@usgs.gov), U.S. Geological Survey
Role: Oversight and performance of satellite transmitter data analysis and modeling

U.S. Fish and Wildlife Service Coordinators

Pete Tuttle (251 928-2037; pete_tuttle@fws.gov) - NRDA

Tom Shearer (361-994-9005, Tom_Shearer@fws.gov) - Kemp's ridley/Endangered Species

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Table 1. Kemp's ridley sea turtle tissue and matrix analyses. Values are the number of samples expected to be analyzed, displayed as X/Y and indicating those collected in 2010 (X) and anticipated in 2011 (Y).

	Samples to Undergo Chemical analyses					Samples to Undergo Biological Analyses					
***Life stage* / Tissue or Matrix**	**Petroleum Comp\ PAH\Petroleum Biomarker**	**PAH / Petroleum Biomarkers**	**PAH metabolites**	**Dispersant**	**HAH Compounds**	**Hatchling sex - gonad histopathology**	**DNA to ID nester / nest relationships**	**Isotopic ratios**	**Clinical Chemistry**	**Cytochrome P450 enzyme activities**	**Cytochrome P450 immunohistochem**
Adult Female Nesters											
Carapace wipes	**1/20**			*							
Blood		**1/20**	**1/20**	*					**0/20**		
Carapace biopsy								**15/45**			
Skin Biopsy							**30/30**				**0/20**
Surviving Hatchlings											
CAMs (pooled by nest)		**48/50**	**68/50**	*	**10/10**					**26/40**	**44/40**
CAMs (individual)										**13/20**	
Hatchling & Late-term Embryo Mortalities											
CAMs										**15/30**	**8/30**
Residual yolk			**39/50**								
Liver										**15/30**	**9/30**
Bile			**56/50**								
Gonad						**722/250**					
Muscle							**340/340**				
Unhatched Eggs											
Pooled egg homogenate		**69/50**	**69/50**	*							
Nests											
Nest-associated sand	**0/20**										

Note: Numbers are current estimates and may change based on field activities and prioritization following the 2011 field season. * - To be determined

Table 2. Loggerhead sea turtle tissue and matrix analyses. Values are the number of samples expected to be analyzed, displayed as X/Y and indicating those collected in 2010 (X) and anticipated in 2011 (Y).

	Samples to Undergo Chemical analyses					**Samples to Undergo Biological Analyses**					
***Life stage* / Tissue or Matrix**	**Petroleum Comp/ PAH/Petroleum Biomarkers**	**PAH / Petroleum Biomarkers**	**PAH metabolites**	**Dispersant screen**	**HAH Compounds**	**Hatchling sex - gonad histopathology**	**DNA to ID nester / nest relationships**	**Isotopic ratios**	**Clinical Chemistry**	**Cytochrome P450 enzyme activities**	**Cytochrome P450 immunohistochem**
Adult Female Nesters											
Carapace wipes	**10/20**										
Blood		**3/20**							**0/20**		
Carapace biopsy											
Skin Biopsy											
Surviving Hatchlings											
CAMs (pooled by nest)		**8/0**	**10/0**							**7/0**	**9/0**
CAMs (individual)										**9/0**	
Hatchling & Late-term Embryo Mortalities											
CAMs											
Residual yolk		**2/0**									
Liver										**1/0**	
Bile		**2/0**									
Gonad											
Muscle											
Unhatched Eggs											
Pooled egg homogenate		**349/50**	**349/50**	*	*						
Nests											
Nest-associated sand	**10/50**										

Note: Numbers are current estimates and may change based on field activities and prioritization following the 2011 field season. * - To be determined