Proposed Data Collection Plan to Assess Injury to Louisiana and Mississippi Estuarine Dolphin Stocks

Points of Contact:
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Reservation: While dolphin tissue samples will be collected under this cooperative agreement, the determination of which analyses are to be conducted and how the results will be used remain subject to further discussion.

I. Natural Resources Being Addressed

Bottlenose dolphins (*Tursiops truncatus*) reside in bays, sounds and estuaries in the northern Gulf of Mexico (Waring et al. 2009) where exposure to oil from the Deepwater Horizon incident is likely to occur. Prior studies of these stocks suggest that there are resident communities, *i.e.*, groups of dolphins that remain in a relatively limited geographic area year-round, over multiple years, and likely over multiple generations (see Waring et al. 2009 for review). Because these estuarine dolphin stocks remain in a restricted area, they are more likely to encounter oil than those marine mammal species that range widely (Wursig 1988).

There are a number of potential routes by which dolphins may be exposed to the oil or associated chemicals. Dolphins may be exposed to volatile organic compounds via inhalation, may incidentally ingest petroleum compounds or dispersants at the surface or in the marine sediments when feeding, may ingest accumulated compounds through their prey, or may be exposed to oil or other chemicals through direct contact with their skin.

This proposal will address potential impacts on northern Gulf of Mexico Bay, Sound and Estuarine stocks of bottlenose dolphins specifically targeting stocks along the Mississippi, Louisiana, and Florida Panhandle coasts. We will collect baseline information on contaminant exposure, reproductive status, and abundance, and then as oil moves into the coastal areas, collect post-impact information on exposure as well as measure endpoints for injury assessment such as calf survival, adult survival and changes in abundance. While we are targeting stocks which are most likely to be exposed and suffer injury, the exposure-response information obtained from this study may be applicable for estimation of risks to other Gulf of Mexico cetacean populations.

Intensive sample and data collection will need to begin immediately in order to obtain baseline (pre-impact) samples and data. In addition, spring (May-July) is a peak calving season for *Tursiops* in many southeastern U.S. coastal and estuarine waters; therefore it is critical to begin photo-documentation immediately to allow for estimation of neonatal survival rates.

II. Purpose/Objectives

The objective of this study is to assess polycyclic aromatic hydrocarbon (PAH) and other contaminant exposure to dolphins associated with the Deepwater Horizon incident and to determine potential effects on fecundity and survival and document changes in abundance. This will require sampling of dolphin tissues to assess contaminant exposure and hormone levels (reproductive and stress hormones), and photo-identification mark-recapture surveys to establish

baseline abundance, prevalence of calves and to identify individual animals to support longitudinal study for survival analysis.
Four areas will be targeted: Chandeleur Sound, LA; Mississippi Sound, MS; Barataria Bay, LA; and St. Joseph Bay, FL (Figure 1). Chandeleur Sound and Barataria Bay will be targeted based on current forecasted oil trajectories. Mississippi Sound and St. Joseph Bay will be targeted due to longer term forecasts for oil movement and because there is historical, long-term information on the bottlenose dolphin stocks in these areas from previous studies by NOAA and partners (Hubard et al. 2004, Balmer et al. 2008). Similar tissue samples will also be collected from Sarasota Bay, FL as part of ongoing dolphin health assessment efforts being conducted by Chicago Zoological Society. The Sarasota Bay samples will allow for comparison with a non-impacted (at least currently) site, but this effort is independent and not included in this proposal.

Historical data/literature. All four areas are considered separate stocks or populations under the Marine Mammal Protection Act (Waring et al. 2009). Photo-identification catalogs and seasonal abundance estimates exist for Mississippi Sound (Hubard et al. 2004) and St. Joseph Bay (Balmer et al. 2008) and may serve for comparison for the proposed studies. However, the abundance estimate for Mississippi Sound is considered out of date (Waring et al. 2009). In addition, previous studies on persistent organic pollutants (polychlorinated biphenyls, organochlorine pesticides, and polybrominated biphenyls) have also been conducted in Mississippi Sound and St. Joseph Bay (NOAA/NIST, unpublished data). There have been no studies of population abundance or pollutant exposure in Chandeleur Sound. Similarly, there are no baseline data available on pollutant exposure for the Barataria Bay population. An abundance estimate for a portion of Caminada Bay/Barataria Bay has been estimated (Miller 2003): 138 – 238 (95% CL 128 – 297) but is also considered out of date.
There are no estimates of fecundity or survival rates for any these regions. However, fecundity and survival rates have been determined for other *Tursiops* stocks for other areas of the Gulf of Mexico and Atlantic coasts and may serve as baselines for comparison (Wells and Scott 1990, Wells 2000, Stolen and Barlow 2003, Wells et al. 2005, Speakman et al. In press).

III. Methods

Contaminant exposure assessment. Tissue samples will be obtained using remote biopsy, a standard mechanism for obtaining tissue samples from free-ranging marine mammals (Gorgone et al. 2008). Biopsy samples are obtained from a small boat using specially designed biopsy darts fired from either a rifle or a crossbow. Photographic identification images of sampled and un-sampled animals will be collected simultaneously with the biopsy sample and compared to existing photo-identification catalogs, where they exist, to provide contextual information on the life-history of sampled animals. The photographs will also provide an opportunity for follow-up of specific individuals. Remote biopsy sampling has been conducted for bottlenose dolphins and a number of other cetacean species in prior studies with no indication of long-term changes in behavior and/or detrimental health effects (Krützen et al. 2006).
The remote biopsy can provide both skin and blubber that can be analyzed for a variety of biological metrics. For this study, skin and blubber samples will be obtained for quantitative polymerase chain reaction (qPCR) analysis to determine CYP1A expression as a biomarker for oil-related contaminant (PAH) exposure. The remaining portion of the blubber sampled will be used for analysis of reproductive and stress hormones, as well as for chemical analysis to determine levels of other contaminants such as polychlorinated biphenyls which may cause

increases in CYP1A but are unrelated to the oil spill incident. Genetic analysis will be conducted on skin samples for sex determination and stock identification. A full depth slice of combined skin and blubber will be used for histopathology and immunohistochemistry to aid in the interpretation of CYP1A qPCR results.

The proposed laboratories to be used for the analysis are listed below; however, the costs for laboratory analysis are not included in this proposal.

- CYP1A analysis: Woods Hole Oceanographic Institute (J. Stegeman and M. Moore)
- Genetic analysis: NOAA Fisheries Marine Mammal Genetics Laboratory (P. Rosel)
- Histopathology and immunohistochemistry: NOAA Fisheries (D. Rotstein)
- Hormone analysis: NOAA Fisheries Southwest Fisheries Science Center (N. Kellar)
- Chemical analysis for additional contaminants: National Institute of Standards and Technology (J. Kucklick)

Assessment of survival, fecundity and changes in abundance. Photo-identification surveys and mark-recapture analysis will be conducted to assess abundance pre- and post-oil exposure and also to identify individuals for estimation of post oil exposure survival and fecundity. Individual animal identification through natural dorsal fin markings is a recognized tool for tracking cetaceans, and specifically bottlenose dolphins, over time (Wells and Scott 1990, Würsig and Jefferson 1990, Wilson et al. 1999). Data obtained from photo-identification surveys can be used to estimate abundance (Wilson et al. 1999, Read et al. 2003, Balmer et al. 2008, Speakman et al. In press) as well as to estimate survival (Speakman et al. In press). In addition, identification and tracking of mother-calf pairs can be used to estimate calf survival (Speakman et al. In press).

Sample Collection/Survey Schedule. Initially, we are proposing to conduct biopsy sampling during 7 May –18 May near Grand Bay and then in Mississippi Sound to obtain samples before oil exposure. We will conduct biopsy sampling in Chandeleur Sound during 24 May - 28 May. Biopsy sampling in St. Joseph Bay will begin the first week of June. This will provide 5-7 days in each site, which we estimate will result in 15-20 samples per site. Following exposure, we would continue sampling at fixed intervals to identify changes in exposure over time. The appropriate intervals for follow-up sampling would need to be determined based on long-term forecast/expectations for oil impacts, but it is estimated that the remote biopsy sampling would need to be repeated monthly. Again, each sampling event would require 5-7 days per site to obtain 15-20 tissue samples. It is estimated that this sampling would need to be continued for at least 2 additional months (see Table 1).
Photo-identification surveys would be initiated immediately and would require 1-2 weeks (~ 9 days) of survey effort per site. Photo-identification surveys would be repeated after 2 months and again after 6 months to acquire data necessary for estimating calf and adult survival as well as post-oil impact abundance estimates.

IV. Data Collection

During photo-identification (photo-ID) surveys, sighting data sheets commonly used during other NOAA photo-ID surveys will be completed for each sighting of dolphins (Appendix 1). These data sheets record information such as location of the dolphin group, how many animals are in the group, estimated age classes, and behavior. Photographs of dolphin dorsal fins will be

processed and catalogued using standard procedures and compared to existing catalogs to obtain as much life history information as possible from individual dolphins. During biopsy operations, a biopsy data sheet (Appendix 2) is completed for every biopsy attempt. We will attempt to photograph the dorsal fin of the individual being sampled to the extent possible. We will also take dorsal fin pictures of other, non-sampled individuals in the group opportunistically. The pictures obtained during biopsy surveys will be processed and cataloged the same way as those collected during photo-identification surveys.

The NOAA NRDA Sampling Spreadsheet will be used to log biopsy samples collected each day and this spreadsheet automatically generates NRDA chain of custody forms. The sample spreadsheets, GPS tracks, and photos of biopsy samples will be uploaded to the NRDA FTP site each evening following the guidance provided by NRDA in a word document titled "Instructions to samplers 2010.05.05." The same files will also be maintained by NOAA NMFS in addition to the photo-identification pictures. All sighting and biopsy datasheets and chain of custody forms will be entered into an electronic database maintained by NOAA/NMFS.

V. Expected Budget and Requested Resources

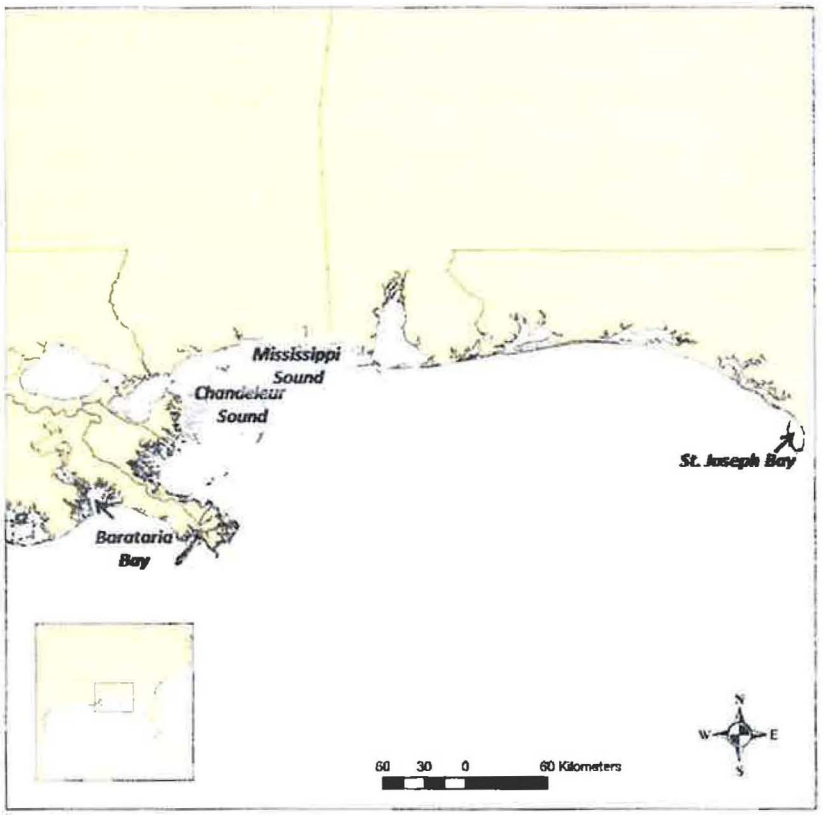
The total cost for the project would be **$619,350**. Budget details are given in attached Excel file. The costs would be split between NOAA and the Chicago Zoological Society (Dr. Randall Wells, [redacted]. NOAA would conduct the studies in the 2 LA and 1 MS site - $484,450 and CZS would conduct the study in St. Joseph Bay, FL - $134,900.

Requested resources: One boat will be available from Pascagoula NMFS laboratory, but an additional small vessel will need to be rented during times that photo-identification and biopsy studies overlap. This is included in the budget detail.

Table 1. Proposed schedule for biopsy and photo-identification surveys.

Study Area/Month	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mississippi Sound								
biopsy sampling	2nd wk	2nd wk	2nd wk					
photo-ID		9 days		9 days				9 days
Barataria Bay								
biopsy sampling	3rd wk	3rd wk	3rd wk					
photo-ID		9 days		9 days				9 days
Chandeleur Sound								
biopsy sampling	4th wk	4th wk	4th wk					
photo-ID		9 days		9 days				9 days
St. Joseph Bay								
biopsy sampling		1st wk	1st wk					
photo-ID		9 days		9 days				9 days
Data processing/ management		X	X	X	X	X	X	X

Figure 1. Map showing planned study areas.



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Appendix 1. Photo-identification data collection sheet.

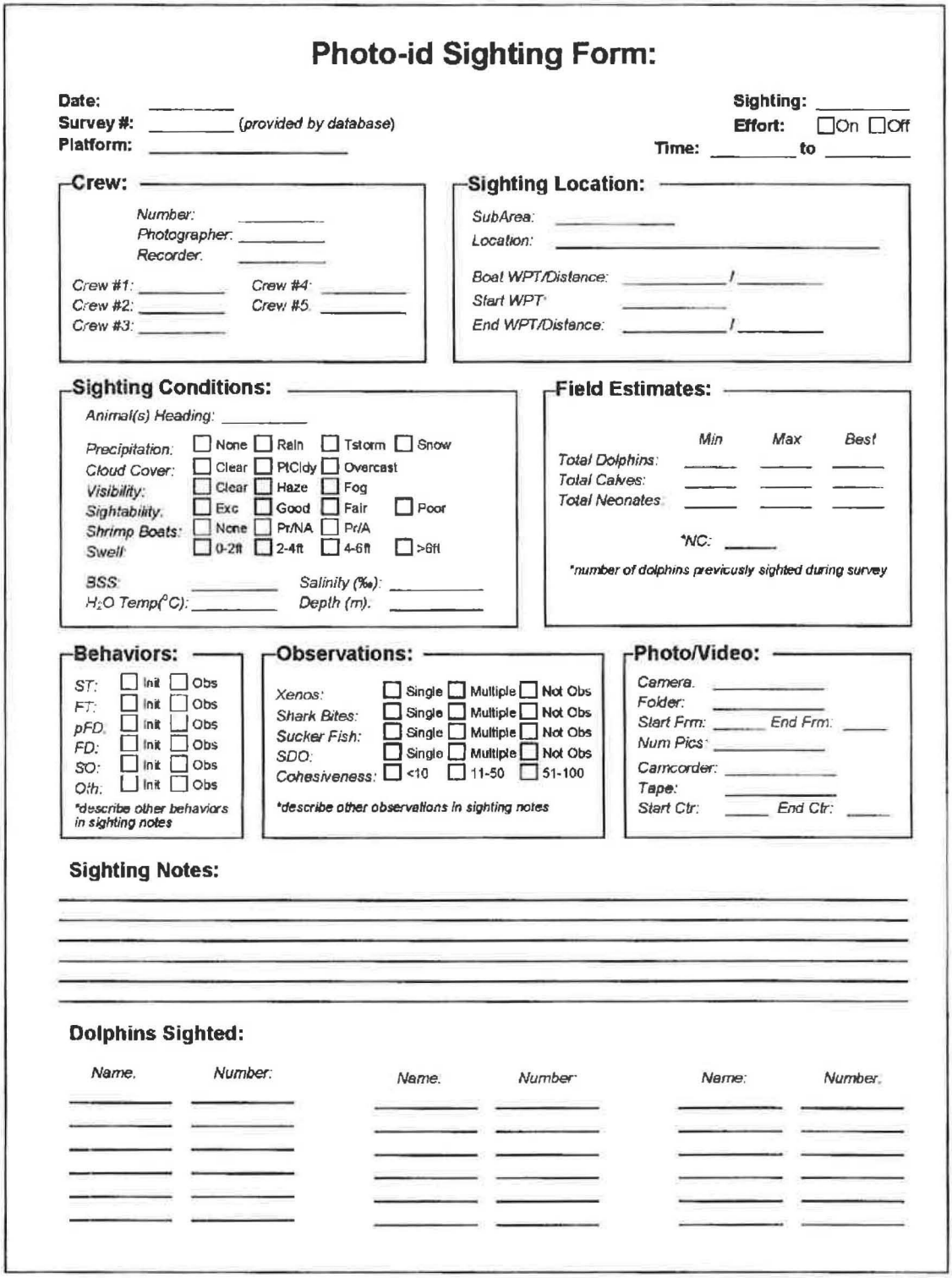


Photo-id Sighting Form:

Date: _____ **Sighting:** _____
Survey #: _____ *(provided by database)* **Effort:** ☐On ☐Off
Platform: _____ **Time:** _____ **to** _____

Crew:

Number: _____
Photographer: _____
Recorder: _____

Crew #1: _____ *Crew #4:* _____
Crew #2: _____ *Crew #5:* _____
Crew #3: _____

Sighting Location:

SubArea: _____
Location: _____

Boat WPT/Distance: _____ / _____
Start WPT: _____
End WPT/Distance: _____ / _____

Sighting Conditions:

Animal(s) Heading: _____

Precipitation: ☐ None ☐ Rain ☐ Tstorm ☐ Snow
Cloud Cover: ☐ Clear ☐ PtCldy ☐ Overcast
Visibility: ☐ Clear ☐ Haze ☐ Fog
Sightability: ☐ Exc ☐ Good ☐ Fair ☐ Poor
Shrimp Boats: ☐ None ☐ Pr/NA ☐ Pr/A
Swell: ☐ 0-2ft ☐ 2-4ft ☐ 4-6ft ☐ >6ft

BSS: _____ *Salinity (‰):* _____
H_2O Temp(°C): _____ *Depth (m):* _____

Field Estimates:

	Min	*Max*	*Best*
Total Dolphins:	_____	_____	_____
Total Calves:	_____	_____	_____
Total Neonates:	_____	_____	_____

**NC:* _____

**number of dolphins previously sighted during survey*

Behaviors:

ST: ☐ Init ☐ Obs
FT: ☐ Init ☐ Obs
pFD: ☐ Init ☐ Obs
FD: ☐ Init ☐ Obs
SO: ☐ Init ☐ Obs
Oth: ☐ Init ☐ Obs

**describe other behaviors in sighting notes*

Observations:

Xenos: ☐ Single ☐ Multiple ☐ Not Obs
Shark Bites: ☐ Single ☐ Multiple ☐ Not Obs
Sucker Fish: ☐ Single ☐ Multiple ☐ Not Obs
SDO: ☐ Single ☐ Multiple ☐ Not Obs
Cohesiveness: ☐ <10 ☐ 11-50 ☐ 51-100

**describe other observations in sighting notes*

Photo/Video:

Camera: _____
Folder: _____
Start Frm: _____ *End Frm:* _____
Num Pics: _____
Camcorder: _____
Tape: _____
Start Ctr: _____ *End Ctr:* _____

Sighting Notes:

Dolphins Sighted:

Name:	*Number:*	*Name:*	*Number:*	*Name:*	*Number:*

Appendix 2. Dart biopsy data collection sheet.

PROJECT # DHOSR2010

Deepwater Horizon Oil Spill Response Biopsy Sheet

DATE _____ (Year Month Day) ☐ Pre-impact ☐ during impact ☐ post-impact

SHOT # _____ TIME _____ (shot) _____ (preservahcm) SIGHTING # _____ HIT MISS (circle one)

SPECIES _____ PLATFORM _____

LATITUDE (in decimal degrees) _____ LONGITUDE (in decimal degrees) _____ RECORDER _____

DEVICE _____ EST. DIST. AT DARTING _____ m SAMPLER _____

SAMPLING HEAD (circle one) F1 F2 F3 F4 G5 DART GUN CHARGE LEVEL (circle one) B G Y R

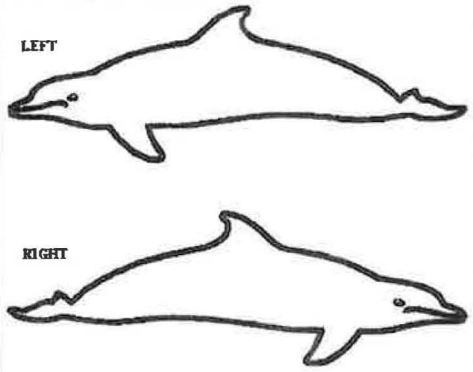
(mark all that apply)

SAMPLE # _____

☐ SKIN – GENETICS - DMSO
☐ BLUBBER – REPRODUCTIVE HORMONES – FROZEN
☐ SKIN & BLUBBER – CYP - FROZEN
☐ BLUBBER-CONTAMINANTS-FROZEN
☐ SKIN & BLUBBER – CYP - FORMALIN
☐ OTHER _____

1 = HEAD	☐	10 = CAUD. P.	☐
2 = PEC. FIN	☐	11 = FLUKES	☐
3 = L. ANT. DF	☐	12 = BELLY	☐
4 = R. ANT. DF	☐	13 = NEAR BLOWHOLE	☐
5 = L. PST. DF	☐	14 = BELOW DF L.	☐
6 = R. PST. DF	☐	15 = BELOW DF R.	☐
7 = GLANCE L.	☐	16 = OTHER	☐
8 = GLANCE R.	☐	17 = DORSAL FIN (DF)	☐
9 = ANT. CAUD. P.	☐	18 = MIDLINE ANT. DF	☐

MARK LOCATION HIT



PHOTOS: YES NO Camera: _____ Frame (start): _____ Frame (hit): _____ Frame (end): _____
Camera: _____ Frame (start): _____ Frame (hit): _____ Frame (end): _____

GROUP SIZE _____ (Adults) _____ (Calves) GROUP BEHAVIOR#: Pre-biopsy _____ Post-biopsy _____

TARGET ANIMAL BIOPSY BEHAVIOR REPONSE* (in chronological order) ☐ ☐ ☐ ☐

LEVEL OF RESPONSE: NONE LOW MODERATE STRONG (circle one) EST. DOLPHIN SIZE _____ m

ANIMALS INCIDENTALLY HARASSED _____ BEHAVIOR RESPONSES* (in chronological order) ☐ ☐

COMMENTS: _____

#* Biopsy behavior responses on back

Draft Preassessment Plan – For Discussion Purposes Only-Not for Release

05-21-2011

Deepwater Horizon/Mississippi Canyon 252
Pre-Assessment and Data Collection Plan – Marine Mammal and Turtle Workgroup

APPROVED:

Department of Interior Trustee Representative: Date

Department of Commerce Trustee Representative: Date

Louisiana Trustee Representative: FOR PEOPLES LIBRARY 5/22/10 Date

Mississippi Trustee Representative: Date

Alabama Trustee Representative: Date

Florida Trustee Representative: Date

BP Representative: Date

05-21-2011

Deepwater Horizon/Mississippi Canyon 252
Pre-Assessment and Data Collection Plan – Marine Mammal and Turtle Workgroup

APPROVED:

Robert W. Ricker — May 21, 2010

NOAA Trustee Representative: Date

Louisiana Trustee Representative: Date

BP Representative: Date

Addendum
Proposed Data Collection Plan to Assess Injury to
Louisiana and Mississippi Estuarine Dolphin Stock ("Addendum")

As set forth herein, this Addendum amends the Proposed Data Collection Plan to Assess Injury to Louisiana and Mississippi Estuarine Dolphin Stock ("Dolphin Plan").

Biopsy samples collected in accordance with the Dolphin Plan shall be analyzed for sex and stock identification. A budget for the sex and stock identification analyses will be submitted and included in this addendum when available. No other biological metrics (including, but not limited to, CYP1A analyses, histopathology, immunohistochemistry, and hormone analysis) or biomarkers shall be analyzed as part of the Dolphin Plan

Notwithstanding this Addendum, each party reserves its right to conduct on its own any biomarker or other laboratory analysis, provided, however, that BP reserves its right to assert that any such analysis does not constitute a reasonable cost of assessment under applicable natural resource damages law.

Approval of this work plan is for the purpose 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 plan.

APPROVAL

NOAA Trustee Representative — Date 6/9/10

Louisiana Trustee Representative — Date

BP Representative — Date 6/9/10

DN: 150691v1

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APPROVAL

NOAA Trustee Representative — Date 6/9/10

Louisiana Trustee Representative FOR LA AMD GUIDRY — Date 6/9/10

BP Representative — Date 6/9/10

DN: 150691v1