

Photographs (see page 2) and morphometrics should be collected for all animals

Chain of custody procedures should be followed for ALL samples, photographs, and datasheets

If the animal is visibly oiled please call 877-433-8299 immediately

**indicates priority sample

LA-492
Field # MC-T-20110325-LAcol

PAH/Chemistry (X2)

Code 2 & 3, 10-20g or 5 ml
Freeze -20, I-chem vial or foil

Blubber
 Liver
 Lung
 Kidney

Freeze -20, I-chem or glass vial:
 **Bile
 **Urine none present
 Whole blood
 Aqueous humor

PAH/Chemistry con't (X2)

Freeze -20, foil, dull side in
All conditions: rated
 **Swab contaminated
 **Tongue depressor (visibly oiled)

Biotoxins: 3-26-11

Freeze -20, codes 2-4, 1 ml or 5g:
 **Feces
 **Urine (if enough after PAH) none present
 Liver

CYP 1A: (X2)

Code 2 & 3, 1 cm thick section.
Freeze, cryovial, -80 if possible
 Skin+full-thick blubber section
Formalin, 10:1 ratio
 Skin+full-thick blubber section

Parasites (codes 2 - 4):

In 70% Isopropyl alcohol
External Representative sample from each species and each location
Internal

Viral: (X2)

Code 2 and early 3: 2 sets - 1 in RNA later vial and 1 in cryovial, both frozen at -80
Code late 3 and 4 - 1 set of priority samples (**) in RNA later if possible

**Brain RNA later -80
Lung assoc LN RNA later -80
**Lung RNA later -80
**Spleen RNA later -80
**Adrenal gland RNA later -80
Kidney RNA later -80
**Liver RNA later -80

Grossly affected Lymph nodes and other grossly affected tissues (list):
no RNA later available

Life History (Codes 2 - 5):

Freeze, -20:
 **Teeth (4 or whole mandible)
 **Skin (isotope)
 **Stomach whole (prey, biotoxin, & PAH) (see stomach instructions page 2)
In DMSO or frozen:
 **Skin (genetics)

Bacterial Culture Swabs or tissues:

Code 2 Ship to lab immediately on ice packs
 lung assoc. lymph node
 **Brain Liver
 Spleen **Lung
Other at vet's discretion:

Histology (code 2 & 3): (X2)

10% neutral buffered formalin, 10:1 formalin:tissue ratio
Samples fixed in formalin should be no more than 1 cm thick

<input checked="" type="checkbox"/> **Lung	<input checked="" type="checkbox"/> **Liver
<input checked="" type="checkbox"/> **Trachea	<input checked="" type="checkbox"/> **Kidney
<input checked="" type="checkbox"/> **Heart (all 4 chambers)	<input checked="" type="checkbox"/> **Adrenal Gland
<input checked="" type="checkbox"/> Aorta	<input checked="" type="checkbox"/> Urinary bladder
<input checked="" type="checkbox"/> Pulmonary Artery	<input checked="" type="checkbox"/> Blubber
<input checked="" type="checkbox"/> Thymus	<input checked="" type="checkbox"/> Skin
<input checked="" type="checkbox"/> **Thyroid	<input checked="" type="checkbox"/> Muscle (specify location)
<input checked="" type="checkbox"/> **Laryngeal Assoc. Lymph. Tissue	<input checked="" type="checkbox"/> Eye (DR) <u>Lin Histo 1</u>
<input checked="" type="checkbox"/> Pharynx	<input checked="" type="checkbox"/> Gonad
<input checked="" type="checkbox"/> Tongue	<input type="checkbox"/> Mammary Gland
<input checked="" type="checkbox"/> Esophagus	<input checked="" type="checkbox"/> **Uterus/Uterine horn
<input checked="" type="checkbox"/> Small Intestine (proximal, mid, distal)	<input checked="" type="checkbox"/> Vagina & Cervix
<input checked="" type="checkbox"/> Colon	<input type="checkbox"/> Prostate & Penis
<input checked="" type="checkbox"/> Pancreas	<input checked="" type="checkbox"/> Lymph nodes (list):
<input checked="" type="checkbox"/> **Spleen	<u>**Thoracic / pre scap</u>
<input checked="" type="checkbox"/> Spinal Cord (specify location)	<u>**Abdominal/Genital</u>
<input checked="" type="checkbox"/> **Brain (cerebrum, cerebellum, stem, pituitary) <u>Histo 1 only</u>	

Live animals:

Blood:
Collect the following and please see preparation information on page 2
1 - 3ml purple top K₃EDTA
5 - 10 ml red/gray tiger top SST tubes
1 red top tube

Freeze -80 in Teflon for PAH:
Whole blood

Swab:
in viral transport media
Blowhole Swab (ship immediately on ice packs if possible. if not, freeze at -80)

Tursiops <115 cm (see also pg 2):

Code 2 early 3 or visibly oiled:
necropsy ASAP by network vet
**Radiograph (before necropsy - dorso-ventral view skull & body)
**Fetus/neonate datasheet
**Skull and skeleton, freeze intact
**Umbilical stump & body wall (formalin)
Serum, from heart, freeze at -20, cryovial

Code Late 3 - 5 (no visible oil):
**Freeze whole (photos, length, PAH swab first)

Pregnant or postparturient females (code 2 & 3):

Uterine horn: Culture swab Formalin/histo -80 RNA later
**Placenta: Culture swab Formalin/histo -80 RNA later
**Amniotic fluid: Culture swab -80 RNA later
Genital LN: Culture swab Formalin/histo -80 RNA later
Fetus: follow fetal/neonate (<115 cm) protocol

Other Samples Collected:		

Standardized external photographs - As feasible, please collect the following from all animals:

Whole body: left side, right side, dorsal, ventral Close ups: Dorsal fin, head, genitals Neonate add: umbilicus, tongue/mouth

Tursiops <115 cm additional details:

Code 2 OR early code 3 OR visibly oiled - any condition: These animals should be necropsied as soon as possible by a stranding network veterinarian. If your vet is unavailable, call the stranding pager and we will help find you an alternative. Please collect dorso-ventral view radiographs of the skull & body before necropsy. Please save the entire skull and skeleton. When collecting the urinary bladder for histo be sure to collect it whole or collect the tip and include the urachus. Late code 3 - 5 (no visible oil): These should be frozen whole. Please collect your external PAH swabs at the stranding site. Please take external photographs and the genetics skin sample in DMSO before freezing. If time allows, please score the external neonate characteristics from the neonatal characters data sheet

Blood preparation instructions - Blood from live animals:

The following blood tubes are for CBC, serum chemistry and serum electrophoresis:

Send 1st samples from intake or beach to Cornell and a pre-release sample to Cornell, other diagnostics may be done locally Send on ice packs the night of collection, if not possible, send the next morning:
 1 - 3ml purple top K3EDTA - refrigerate, send whole on ice packs
 1 - 10 ml red/gray tiger top SST tube - spin and refrigerate, send on ice packs

The following blood tubes are for hormones and can be shipped to cornell in batches:

(hormones include: cortisol, progesterone, testosterone, aldosterone and thyroid hormones - total T4, free T4 and total T3)
 2 - 10 ml red/gray tiger top SST tubes - spin and remove the serum from both into a 5 ml cryovial, freeze at -80, ship on dry ice

Serum for archive:

1 - 10 ml red/gray tiger top SST tube - spin, remove serum into a cryovial and archive at -80

Serum for biotoxin:

1 - 10 ml red/gray tiger top SST tube - spin, remove serum into a cryovial, freeze at -20 until shipment on dry ice

Buffy coat (if possible):

1 red top tube, spin, carefully remove buffy coat into a cryovial and freeze at -80

Blood preparation instructions - Blood from dead animals:

Tursiops calves <115 cm - code 2 : Use red/gray tiger top SST tubes, collect as much blood as possible from a heart stick - spin, remove serum into a cryovial, freeze at -20 for future shipment, will be used for electrophoresis
 Code 2 or 3 - please collect whole blood in an i-chem or glass vial and freeze at -20 for PAH

Stomach special instructions:

Tie off the stomach at both ends. Remove whole stomach and freeze in plastic. Do not open it! A person will be identified who will receive the stomachs, examine them for internal lesions, collect histology samples of lesions as needed, identify the prey and subsample for biotoxins

PAH/Chemistry:

If you have questions regarding collection methodology, please see attached Appendices 6 & 7 from the Marine Mammal Oil Spill Response Guidelines