APPENDIX H

GENERAL DESCRIPTIONS OF RESEARCH METHODOLOGIES UNDER THE ESA/MMPA PERMIT

Many public comments on the draft PEIS were specific to the methodologies addressed in this Appendix. In several areas, revisions were included below. For more specifics on how public comments were addressed, please refer to Appendix N of this PEIS.

1. Current ESA/MMPA Permit Activities

The activities described in this Section are those that may be conducted under the current ESA/MMPA permit issued to the Marine Mammal Health and Stranding Response Program. Many of the activities are only applicable to the scientific research conducted by Co-Investigators under the permit. Some activities are also applicable to the emergency response of ESA-listed species, which is covered under the ESA/MMPA permit. This section does not include information on basic stranding response activities.

1.1.1 Close Approach

Animals may be taken through close approaches by aircraft for disentanglement, photo-identification, behavioral observation, hazing (during emergency response), and incidental harassment. Animals may be taken through close approaches by vessel for disentanglement, photo-identification, behavioral observation, capture, tagging, marking, biopsy sampling, skin scrapes, swabs, collection of sloughed skin and feces, breath sampling, blood sampling, administration of drugs, video recording, hazing (during emergency response), and incidental harassment. More than one vessel may be involved in close approaches and vessels may approach an animal more than once, in order to complete research tasks. Incidental harassment of non-target animals may occur during close approaches by aircraft or vessel. During emergency response and research activities, close approaches may occur for any age class, sex, and species (including ESA-listed species).

1.1.2 Aerial Surveys

Aerial surveys are used to: locate imperiled marine mammals (ESA-listed and non-listed species); monitor behavior or disease in a given population or individual; survey the extent of disease outbreaks or die-offs; and locate carcasses. During emergency response and research activities, aerial surveys may occur for any age class, sex, and species (including ESA-listed species).

The aircraft type used during emergency response activities depends upon the aircraft available at the time of the response and the logistics of the activity. Aircraft type includes helicopters and fixed-wing aircraft. The frequency of surveys is dependent on the circumstances of the involved stranded or entangled animals, the disease, or the occurrence of an Unusual Mortality Event (UME). Aerial

surveys are flown along predetermined transect lines at a set altitude and air speed while observers scan the water for signs of marine mammals.

The speed and altitude of the aircraft depends on the aircraft and the response or research situation. For large cetaceans, surveys would be flown at an altitude of 230-300 m (750-1,000 ft) at approximately 110 knots (203 km/hr). For right whales, surveys would be flown at 100 knots (185 km/hr). For smaller cetaceans, surveys would be flown at an altitude of approximately of 230 m (750 ft). Large survey aircraft would be flown at 110 knots (203 km/hr) and small aircraft would be flown at 97 knots (179 km/hr). When an animal or group of animals is sighted, the survey aircraft descends and circles over the animal or animals to obtain photographs and assess the animal, if necessary.

A minimum altitude of 153 m (500 ft) would be used for pinniped surveys. The typical altitude would be between 182-244 m (600-800 ft) at 80 to 100 knots (148-185 km/hr). For Steller sea lion surveys during the breeding season, an altitude of at least 214 m (700 ft) would be used to collect photographs. In the non-breading season, surveys would be flown between 150-200 m (492-655 ft) at a speed of 100-150 knots (185-278 km/hr). All aerial surveys will be flown according to the NOAA Aviation Safety Policy (NOAA Administrative Order 209-124), with trained observers and pilots.

1.1.3 Vessel Surveys

Vessel surveys of both ESA-listed and non-listed marine mammals may be conducted to: collect data on animal abundance; assess animals; locate animals for research activities; and collect research samples. The vessels themselves may be used as a platform for conducting animal sampling. Vessel surveys may be used to monitor animals subsequent to capture-release sampling for assessment, photo-identification, and tracking.

For small cetaceans, inshore monitoring surveys are conducted using small (5-7 m) outboard motor powered boats. Animals are located by having crew members visually search waters as the boat proceeds along a specified route at slow speeds (8-16 km/hr). Animals outfitted with Very High Frequency (VHF) radio tags are located by listening for the appropriate frequency and, after detecting a signal, maneuvering the boat towards the animal using a combination of signal strength and directional bearings. Frequencies and remote sensors may also be monitored. Once a group of animals is located, the boat approaches the group so that crew members can assess their physical and medical condition. Photographs of the dorsal fins of individual animals are taken for later identification and matching to existing dorsal fin catalogs. When an animal is located that has been recently caught for a health evaluation, an attempt is made to photograph the dorsal fin and body to

confirm identification, health, position, and behavior. A photograph of the dorsal fin would also be used to assess wound healing from tag attachment. The area behind and below the posterior aspect of the dorsal fin may also be photographed to assess biopsy wound healing. A telephoto lens would be used for photographs, so vessels would not need to be too close to animals.

Multiple approaches may be required to obtain appropriate quality photographs, particularly if there are multiple individuals within a group. Close approach is terminated and the boat moves away from the group if animals begin to display behavior that indicates undue stress (e.g., significant avoidance behavior such as chuffing [forced exhalation], tail slapping, or erratic surfacing).

1.1.4 Hazing

Hazing of ESA-listed marine mammals may occur if an animal is in the vicinity of an oil or hazardous material spill, harmful algal bloom, sonar, or other harmful situations. Animals may also be hazed to deter a potential mass stranding. For all marine mammals, including threatened and endangered species, hazing is authorized under the MMHSRP's MMPA/ESA permit. Hazing methods include, but are not limited to, the use of acoustic deterrent devices, acoustic harassment devices, visual deterrents, vessels, physical barriers, and capture and relocation. The correct use of deterrents incorporates the element of surprise, while minimizing the potential for habituation.

Acoustic deterrents that may be used to deter cetaceans include, but are not limited to: pingers, bubble curtains, Oikomi pipes, acoustic harassment devices (e.g., Airmar devices), seal bombs, airguns, mid-frequency sonar, low-frequency sonar, predator calls, and aircraft. Pingers, which are typically used in the commercial fishing industry, produce high-frequency pulses of sound to deter animals. The standard pinger emits a signal of 10 kHz (with harmonics to at least 60 kHz) with a source level of 132 dB re µPa at 1 m, which is within the hearing range of most cetaceans (Reeves *et al.* 1996). Bubble curtains may be used as a barrier from other acoustics. Oikomi pipes are banged together by personnel on boats. They have been effective in herding cetaceans, but may not be as effective in keeping animals out of a large area.

Airmar devices have a source level of 195 dB re µPa and their peak energy is at 10 kHz with higher harmonics. These devices may be moved at low speeds on small boats or may be hull mounted on boats to allow faster movement. They may be able deter animals 3 km away. A line of directional Airmar devices could be deployed at the sight of a spill of near cetaceans to move them away. The received levels needed to cause deterrence without acoustic trauma are unknown.

Seal bombs are explosive devices that are weighted with sand to sink and explode at 2-3 m underwater, producing a flash of light and an acoustic signal of less than 2 kHz and a source level of approximately 190 dB. The noise and light would potentially startle marine mammals, but not cause any injuries (Petras 2003). Airguns are generally a towed array that is deployed behind a ship. Their peak energy is dependent on size, and may range from 10 Hz to 1 kHz. Airguns produce broadband pulses with energy at frequencies ranging over 100 kHz. The higher frequencies are less intense and attenuate faster. Harbor porpoise have been seen moving away from airguns 70 km away.

Mid-frequency sonar may be used to deter cetaceans. It has caused deterrence in killer whales in Haro Strait during the 2003 USS Shoup transit episode. The sonar had a source level of approximately 235 dB (exact level is classified) and the frequency ranged from 2.6-3.3 kHz over 1-2 second signals emitted every 28 seconds (USN 2004). Mid-frequency sonar could be effective over 25 km, which would be important for deterring animals during a large oil spill. Low-frequency sonar may also be used, but may too low for some cetaceans to hear.

Predator calls (typically killer whale calls) may be played to deter potential prey. However, in most situations, predator calls have proven ineffective in changing prey behavior. Aircraft, such as helicopters, generate a fair amount of noise and wave movement at close range and could produce a startle or avoidance response. This may be effective initially, but animals would likely habituate quickly. Aircraft could also be used to deploy seal bombs, if necessary. Vessels may be used to herd animals back out to open water or away from a hazardous situation. Booms or line on the water may be used to displace small odontocetes from stranding. Fire hoses may be used at close range as a physical deterrent, although their effectiveness is not known.

Pinniped acoustic deterrents include seal bombs, Airmar devices, predator calls, bells, firecrackers, and starter pistols. Visual deterrents for pinnipeds include flags, streamers, and flashing lights. Exclusion devices for pinnipeds may include nets or fencing.

1.1.5 Capture and Restraint

Capture of marine mammals may be necessary during research and enhancement activities to collect specimens; perform an examination; evaluate wound, disease, entanglement, or injury; or attach tags and/or scientific instruments. Capture of non-ESA listed marine mammals would be necessary during research activities. During emergency response, these activities may occur for any age class, sex, and species (including ESA-listed species). For research activities, capture, restraint, and handling would occur on all animals except for young of the year.

Capture methods include, but are not limited to, nets, traps, behavioral conditioning, and anesthesia/chemical immobilization. These procedures would be performed or directly supervised by qualified personnel and, if possible, an experienced marine mammal veterinarian would be present to carry out or provide direct on-site supervision of all activities involving the use of anesthesia and sedatives. Capture and restraint methods for pinnipeds and cetaceans are discussed below.

1.1.5.1 Pinniped Capture and Restraint

Capture and restraint of pinnipeds occurs during health assessment studies, emergency response, and disentanglement activities. Pinnipeds may be captured on land or in water by various methods, depending on the targeted age classes. On land, pups (>5 days to 2 months old) and juveniles (>2 months to 3 years old) may be captured by hand. Juveniles and adults (>3 years old) may be captured using circle, hoop, dip, stretcher, and throw nets. Net guns and pole nooses may be used for capture of pinnipeds. An injectable immobilizing agent, administered remotely by a dart, may also be used to subdue older animals. Herding boards may be used to maneuver animals into cages. For water captures of pinnipeds, dip nets, large nets, modified gill nets, floating or water nets, and platform traps may be used. Purse seine nets may be used offshore of haul-out sites to capture animals when they stampede into the water (Jeffries et al. 1993). Animals become entangled by the net as it is pulled ashore. Once removed from the net, animals are placed head first into individual hoop nets. Pups may be restrained by hand, in a hoop net, or with the inhalation of a gas anesthesia (administered through a mask over their nose). Older animals may be restrained using gas anesthesia (administered through an endotracheal tube), a fabric restraining wrap, a restraining net, or through sedation (either intramuscular (IM) or intravenous (IV)).

An animal would not be manually restrained for more than 30 minutes. Procedures would be conducted as quickly as possible to reduce stress on the animal. Vital signs, including respiration, heart rate, and temperature, would be continuously monitored and recorded at the start of handling and every 5 minutes thereafter.

1.1.5.2 Cetacean Capture and Restraint

Capture and restraint of cetaceans occurs during health assessment studies, emergency response, and disentanglement activities. Typical methods currently used during health assessment studies and for emergency response are described below. However, these methods may vary depending on the species and location. All capture and restraint protocols would be approved by NMFS PR1 before their use. For health assessment studies of small cetaceans, small schools of animals are approached

for identification (see description under vessel surveys). If the school contains animals desired for capture, the school is followed until it is in waters that facilitate safe captures (waters outside of boating channels, equal to or less than 1.5 m deep, where currents are minimal). Typically no more than three animals are captured at one time. The animals are encircled with a 600 m long by 4 m deep seine net, deployed at high speed from an 8 m long commercial fishing motor boat. Small (5-7 m) outboard-powered vessels are used to help contain the animals until the net circle is complete. These boats make small, high-speed circles, creating acoustic barriers.

Once the net is completed, about 15-25 handlers are deployed around the outside of the corral to correct net overlays and aid any animals that may become entangled in the net. The remaining 10-20 or more team members prepare for sampling and data collection and begin the process of isolating the first individual. Isolation is accomplished by pinching the net corral into several smaller corrals. Handlers are usually able to put their arms around the selected animal as it bobs in place or swims slowly around the restricted enclosure. However, a few animals may strike the net and become entangled. After animals are restrained by handlers, an initial evaluation is performed by a trained veterinarian. Once cleared by the veterinarian, the animal is transported to the processing boat via a navy mat and/or a sling. A sling is also used to place an animal back in the water for release.

In some cases, cetaceans may need to be captured in deep waters. A break-away hoop-net is used to capture individuals as they ride at the bow of the boat. When they surface to breathe, the hoop is placed over their head and they move through the hoop, releasing the net. The additional drag of the net slows the animals substantially, but the design allows the animal to still use its flukes to reach the surface to breathe. The net is attached to a tether and large float, and the animal is retrieved, maneuvered into a sling and brought onboard the capture boat.

For emergency response, small cetaceans in shallow water may be caught using a net deployed from a boat with methods similar to those described above. In rivers and canals, responders may use their bodies to herd an animal and then hand catch it. In deep water, hoop net may be used to capture animals.

1.1.6 Transport

Vehicles, boats, or aircraft are used to transport marine mammals to rehabilitation facilities or release sites. Cetaceans may be transported on stretchers, foam pads, or air mattresses. For short-term transport, closed-cell foam pads are preferred because they are rigid and do not absorb water. Open cell foam is typically used for long-term transport of cetaceans because it can contour to the animal's

form. Boxes may be constructed to transport the animal upright in a stretcher. Cetaceans must be protected from exhaust fumes, sun, heat, cold, and wind, as transport often occurs on the flatbed of a truck. Animals are kept moist and cool, to avoid overheating (Geraci and Lounsbury 2005).

Small pinnipeds are typically transported in plastic kennel cages. Cages are large enough for animals to turn around, stretch out, and raise their heads. Cages should prevent animal contact with waste and allow proper air circulation. As with cetaceans, pinnipeds traveling by vehicle must be protected from the sun, heat, cold, wind, and exhaust fumes. Pinnipeds may overheat during transit and wetting the animal helps to prevent hyperthermia (Geraci and Lounsbury 2005). Large pinnipeds may need to be sedated during transport. Sedation of large pinnipeds would be performed or directly supervised by qualified personnel and, if possible, an experienced marine mammal veterinarian would be present to carry out or provide direct on-site supervision of all activities involving the use of sedatives.

Transport procedures for marine mammals under U.S. jurisdiction follow the Animal and Plant Health Inspection Service's "Specifications for the Humane Handling, Care, Treatment, and Transportation of Marine Mammals" (9 CFR Ch 1, Subpart E). The "Live Animal Regulations" published by the International Air Transport Association (IATA), and accepted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora, are followed for the air transport of animals under foreign jurisdiction (IATA 2006). Both sets of standards have specifications for containers, food and water requirements, methods of handling, and care during transit.

1.1.7 Tagging/Attachment of Scientific Instruments

Tagging of ESA-listed marine mammals may be used to monitor an animal's movements after immediate release (from a stranding site), release after rehabilitation, or release after research activities. Tagging of non-listed marine mammals may occur as part of a research project or for monitoring rehabilitated animals post-release when such tag devices are considered intrusive or experimental. Other tags or scientific instruments may be used to obtain data on dive depth, dive time, water temperature, light levels, and animal and other underwater sounds. During emergency response activities, tags or scientific instruments may be attached to any age class, sex, and species (including ESA-listed species). During research activities tags will not be attached to large cetacean calves less than six months of age or females accompanying such calves. For small cetaceans, no tagging will occur on calves less than one year of age.

A variety tags (including scientific instruments) may be attached to or implanted in an animal. The type of tag and method of attachment depends on the species being tagged and the research or

question being addressed. Types of tags that are used include, but are not limited to: roto-tags (cattle tags), button tags, very high frequency (VHF) radio tags, satellite tags, Passive Integrated Transponder (PIT) tags, D-tags, code division multiple access (CDMA) tags, pill (e.g., stomach temperature telemeters), time-depth recorders (TDRs), life history transmitters (LHX tags), and crittercams (video cameras). Tag attachment methods vary with tag type, species, and circumstances. Attachment methods for cetaceans include, but are not limited to: bolt, buoy, punch, harness, suction cup, implant, or ingestion. Pinniped attachment methods include, but are not limited to: glue, bolt, punch, harness, suction cup, surgical implant, or ingestion. Specific tags and methods of attachment will be evaluated for each situation.

1.1.7.1 Tagging of Cetaceans

Tags are generally attached to free-swimming cetaceans by crossbow, compound bow, rifles, spear guns, slingshot (or throwing device), pole or jab spears. Tags will only be applied by experienced marine mammal biologists. Prior to deployment, tag type and attachment method will be approved by NMFS PR1. Attachments are temporary and occur via a suction cup device or implant. Scientific instruments attached to suction cups include, but are not limited to D-tags, TDRs, VHF tags, satellite tags, and crittercams. Large, slow moving whales may be tagged via suction cups using a pole delivery system, cantilevered on the bow of a boat. Bow-riding animals may be tagged using a hand held pole. Crossbows are the preferred method for tagging fast-moving toothed whales. Tags are attached on the dorsal surface of the animal behind the blowhole, closer to the dorsal fin. Tag placement ensures that the tag will not cover or obstruct the whale's blowhole, even if the cup migrates after placement (movement would be toward the tail).

Implantable tags may be attached in free-swimming cetaceans by mounting the instrument on an arrow tip or other device designed to penetrate the skin of the animal. Tags would typically be attached by crossbow and may include, but not limited to satellite tags, VHF tags, and TDRs. Buoys are used to attach VHF or satellite tags to gear on entangled whales. Buoys may also be attached to increase drag in an attempt to slow a whale for disentanglement.

For animals in hand, tags may be attached for longer deployments. Roto-tags may be attached to cetaceans with a plastic pin to the trailing edge of the dorsal fin. Button tags are plastic disks attached with a bolt through the dorsal fin. VHF tags (roto-radio tags) may also be bolted through the trailing edge of the dorsal fin. The bolts on each type of tag are held in place by magnesium nuts that will corrode in seawater and allow the tag to be released.

Satellite or VHF tags can be mounted on a molded plastic or fabric saddle that would be bolted through the dorsal fin (Geraci and Lounsbury 2005) or dorsal ridge. Plastic saddles would be padded with foam on the inside to reduce skin irritation. Saddles will be attached to the dorsal fin with two or three Delrin pins secured with magnesium nuts. The nuts would corrode in seawater, allowing the package to be released within a few days or weeks. The saddle will be raised off the surface of the dorsal fin by inserting foam washers on the pins between the skin and saddle. Two washers would be used to provided approximately 6 mm of separation

Dorsal ridge "spider tags" may be used on beluga whales (NMFS Permit No. 782-1719) (Litzky *et al.* 2001). Up to four holes are bored in the region of the anterior terminus of the dorsal ridge using a coring device (trochar) with a diameter of no more than 1 cm. Each insertion and exit point for the trochars would be prepared by cleaning with an antiseptic wipe, or equivalent. Rods of nylon or other non-reactive material, not greater than 1 cm in diameter and 50 cm in length, would then be pushed through the holes and attached to the wire cables or fabric flange or straps of the satellite tags or through bolt holes in the tag. The wire cables would be tightened to hold the tag against the back of the animal to minimize tag movement and drag, but would not be put under significant tension to avoid pressure necrosis around the pin insertion points. The other attachment systems would be manipulated to achieve the best possible fit depending on their design. Excess rod would then be cut off. All equipment would be sterilized in cold sterile solution, alcohol, or equivalent, and kept in air- and water-tight containers prior to use. Trochars and rods would be coated with antiseptic gel prior to insertion and each trochar would only be used for one hole before it is cleaned, sharpened, and re-sterilized. Where more than one instrument is to be attached, the number of pins would be limited to four.

1.1.7.2 Tagging of Pinnipeds

A fast drying epoxy adhesive is used to glue scientific instruments to pinnipeds. Instruments may be attached to the dorsal surface, head, or flippers and will release when the animal molts. Roto-tags can be attached to flippers using a single plastic pin. Tags can also be surgically implanted into the body cavity or muscle of pinnipeds. Implanted tags include PIT and LHX tags.

A PIT tag is a glass-encapsulated microchip, which is programmed with a unique identification code. When scanned with an appropriate device, the microchip transmits the code to the scanner, enabling the used to determine the exact identity of the tagged animal. PIT tags are biologically inert and are designed for SQ injection using a syringe or similar injecting device. The technology is well

established for use in fish and is being used successfully on sea otters (Thomas et al. 1987), manatees (Wright et al. 1998), and southern elephant seals (Galimberti et al. 2000). PIT tags are also commonly used to identify domestic animals. PIT tags may be injected just below the blubber in the lumbar area, approximately 5 inches lateral to the dorsal midline and approximately 5 inches anterior to the base of the tail. Tags may also be injected at alternative sites on a pinniped's posterior, but only after veterinary consultation. The injection area would be cleansed with Betadine (or equivalent) and alcohol prior to PIT tag injection. PIT tags are currently being used in Hawaiian monk seals (NMFS Permit No. 848-1695).

LHX tags are implantable, satellite-linked life history transmitters used to measure mortality events in pinnipeds. The tag allows continuous monitoring from up to five built-in sensors, including pressure, motion, light levels, temperature, and conductivity. The tag is surgically implanted into the abdominal cavity while the animal is anesthetized. An incision of 7-8 cm long through the abdominal wall, including abdominal muscles and peritoneal layers, is required to insert the tag. The incision is closed using absorbable sutures and may be further secured with surgical glue or dissolvable staples. When the animal dies, the tag is released from the body and floats to the surface or falls out onshore. Data from the tag is transmitted via the ARGOS system to a NOAA satellite. The battery life of an LHX tag is well over five years. LHX tags are being evaluated under current NMFS PR1 research permits (Permit No.1034-1685 [California sea lions] and No. 881-1890 [Steller sea lions]).

1.1.8 Marking

Marking methods for marine mammals during emergency response and research activities include, but are not limited to: bleach, crayon, zinc oxide, paint ball, notching, and freeze branding. Hot branding will not be used as a marking method. Crayons, zinc oxide, and paint balls can be used on cetaceans and pinnipeds for temporary, short-term marking. Bleach or dye (human hair dye) markings can be used on pinnipeds. The marks are temporary, with the length of time dependent on molting. Notching can be used to permanently mark cetaceans by cutting a piece from the trailing edge of the dorsal fin. Notching in pinnipeds removes a piece of skin from the hind flipper of phocids (true or earless seals) and the foreflipper of otariids (sea lions and fur seals).

Cetaceans can be marked using freeze branding, typically on both sides of the dorsal fin and/or just below the dorsal fin. Freeze branding is used during health assessment studies to mark all animals for post-release monitoring. Freeze branding uses liquid nitrogen to destroy the pigment producing cells in skin. Each brand (typically 2" numerals) is supercooled in liquid nitrogen and applied to the dorsal

fin for 15-20 seconds. After the brand is removed, the area is wetted to return the skin temperature to normal. During health assessments, each animal is photographed and videotaped to record the locations of freeze brands. Brands will eventually re-pigment, but may remain readable for five years or more. Freeze brands provide long-term markings that may be important during subsequent observations for distinguishing between two animals with similar fin shapes of natural markings.

Freeze branding may be used to produce two types of marks on pinnipeds. Short contact by the branding iron destroys pigment producing cells, leaving an unpigmented brand. Longer contact with the brand destroys these cells and the hair, leaving a bald brand (Merrick *et al.* 1996). Hot branding of pinnipeds will not be conducted during permit activities.

1.1.9 Disentanglement

Disentanglement efforts are conducted for many marine mammals. For large whales, disentanglement efforts may include vessel and aerial surveys for the affected animal and incidental harassment of non-entangled animals during these searches. Close approaches may occur to assess the extent of the entanglement and the health of the animal. The animal may be either physically or chemically restrained. Physical restraint of the animal may be used to slow down an animal, provide control, and maintain large whales at the surface. Physical restraint is accomplished by attaching control lines, floats, buoys, and/or sea anchors to the entangling gear with a grappling hook or by attaching new gear to the animal to hold it. The drag from small boats may also slow down an animal. Remote sedation may also be used to restrain the animal. Animals may be tagged with telemetry buoys to monitor their location. Responders use control lines to pull themselves up to the whale. Cutting of lines and possibly flesh (when the line is embedded) may occur during disentanglement. Biopsy sampling may occur, either through the use of a remote dart (described below under biopsy sampling) or the collection of tissues from the removed fishing gear. If the injuries from an entanglement appear to be life-threatening, the animal may be euthanized. NMFS and marine mammal experts would be consulted before deciding to euthanize a large whale. Euthanasia techniques are discussed later in this application. A necropsy would be performed and the carcass would be properly disposed.

Disentanglement efforts for small cetaceans may include capture with incidental disturbance of non-entangled animals, restraint, surgery, rehabilitation, administration of chemical agents (sedatives and/or antibiotics), and release. Response to entangled small cetaceans typically requires in-water capture of free-swimming animals. Some animals may have impaired locomotion if the gear is heavy

or anchored. Capture methods for small cetaceans are described above. If the injuries from an entanglement appear to be life-threatening, the animal is not likely to make a recovery on its own, or if the animal is afflicted with a potentially treatable illness or infection, it may be placed in rehabilitation. If rehabilitation space is not available, the animal would be euthanized. A necropsy would be performed and the carcass would be properly disposed.

An entangled pinniped would be selected for capture if: 1) the entanglement or injury impedes feeding, swimming, or ambulation; 2) the gear is unlikely to fall off on its own; 3) the animal is likely to "grow" into the gear, causing constriction; 4) the gear is cutting into the flesh or likely to cut into the flesh into the future; 5) the injury appears life-threatening or infected, or likely to become infected; or 6) the benefits of capturing and disentangling or collecting the animal for rehabilitation outweigh the risks to the animal and the herd. Entangled pinnipeds are typically captured on land when they are hauled out. Capture methods for pinnipeds are described above.

Disentanglement of pinnipeds may be achieved by simply cutting off the gear. A variety of instruments, including shielded knives, bandage scissors, wire cutters, and dog nail clippers may be used to safely accomplish this task. For emergency situations (e.g., entangled animals anchored in the water) or if the situations allows, long-handled, shielded knives can be used to cut off netting from a distance. The attending veterinarian (or other qualified individual) will determine which instrument(s) is appropriate for the situation. Once the gear is removed, it is photographed, measured, and retained for submission to NMFS. The wound (if any) is cleaned thoroughly by flushing with copious amounts of an appropriate disinfectant and treated with a topical antiseptic cream. An animal may be freed of gear and immediately released, or brought into a rehabilitation facility for a period of time prior to release. Every disentangled animal (except those that are not restrained) are tagged with: a roto-tag on the rear flipper; a head tag glued to the fur or marked; and/or paint stick markings for post release monitoring. Satellite tags maybe considered for healthy animals, weighing 75 lbs or more, if supplies and experienced personnel are available. Methods for tagging are described above.

If the pinniped will be immediately released after disentanglement, the following data will be collected (as feasible): straight length; sex; weight estimate; photographs of the animal, wound (if any), and gear; general locations; and GPS coordinates. Alert animals would be released from the original capture site unless conditions dictate otherwise. Animals would not be released near high drop-offs, heavy boat traffic, heavily human populated beaches, or obvious hazards. The attending veterinarian (or qualified individual) will direct the removal of restraint devices and withdrawal of the animal for a safe release. Crowder boards would be placed between the animal and the water, to

prevent the animal from fleeing into the water before the capture net has been removed. Once the animal has completely freed itself from the capture net, the crowder boards would be opened to allow access to the water. The animal would retreat to the water at is own pace.

An animal may be placed into rehabilitation if the injuries appear to be life-threatening, it is not likely to make a recovery on its own, or if it is afflicted with a potentially treatable illness or infection. Transport methods are described above. If rehabilitation space is not available, the animal would be euthanized. A necropsy would be performed and the carcass would be properly disposed.

1.1.10 Sample Collection and Analysis

Specimen samples would be taken from ESA-listed species during both research and enhancement (i.e., stranding/entanglement response) and from non-listed species during intrusive research [the Order Cetacea and the Order Pinnipedia (except walrus)]. Specimen materials may include, but are not necessarily limited to: earplugs, teeth, bone, tympanic bullae, ear ossicles, baleen, eyes, muscle, skin, blubber, internal organs and tissues, reproductive organs, mammary glands, milk or colostrums, serum or plasma, urine, tears, blood or blood cells, cells for culture, bile, fetuses, internal and external parasites, stomach and/ or intestines and their contents, feces, air exhalate, flippers, fins, flukes, head and skull, and whole carcasses. Specimens may be acquired opportunistically with ongoing studies or prospective design plans; therefore specific numbers and kinds of specimens cannot be predetermined. Because all specimens will be acquired opportunistically, the MMHSRP will have minimal control over the age, size, sex, or reproductive condition of any animals that are sampled. During research activities, samples would not be collected from young of the year animals. Specific methods for biopsies, blood, breath, ultrasound, and other sampling are described below under the corresponding section.

Marine mammal specimens collected for analysis or archiving would be legally obtained from the following sources:

1. On-going live animal capture/release research programs authorized by this permit or under separate permit of other researchers;

2. Live animal capture/release as part of a stranding response, disease, emergency response, or die-off investigation of ESA-listed marine mammals in the U.S., and any marine mammal species abroad;

3. Live ESA-listed animals stranded or in rehabilitation in the U.S. [and from any marine mammal species abroad stranded or in rehabilitation];

4. Captive animals (public display, research, or rehabilitating), when sampling is beyond the scope of normal husbandry or normal rehabilitation practices (i.e., intrusive research on ESA-listed or non-listed species);

5. Captive public display or research animals during normal husbandry or other permitted research;

6. ESA-listed marine mammals found dead on the beach or at sea in the U.S.; and any marine mammal species found dead on the beach or at sea in a foreign country/waters.

7. Animals directly taken in fisheries in countries where taking of such animals is legal;

8. Animals killed during subsistence harvests by native communities;

9. Animals killed incidental to recreational and commercial fishing operations;

10. Animals killed incidental to other human activities;

11. ESA-listed marine mammals found dead as part of NOAA investigations in the U.S. (*e.g.* harmful algal blooms, oil spills, etc.);

12. Soft parts sloughed, excreted, or discharged by live animals (including blowhole exudate);

13. Live animals during disease surveillance;

14. Bones, teeth, or ivory of ESA-listed species found on the beach or on land within ¼ mile of the ocean;

15. Confiscated animals (*e.g.*, as part of enforcement action); or

16. Animals legally taken in other permitted research activities in the U.S. or abroad.

Specimen and data collection from marine mammal carcasses may follow the necropsy protocols for pinnipeds (Dierauf 1994), right whales (and other large cetaceans) (McLellan *et al.* 2004), killer whales (Raverty and Gaydos 2004), small cetaceans (HSWRI 2005) and all marine mammals (Pugliares *et al.* 2007). These include how samples would be stored, transported, and analyzed. During live animal response or research, specimen and data collection protocols would depend on the samples being collected and the intended analyses. All sample analyses occur at various diagnostic laboratories in the U.S. and abroad.

1.1.11 Biopsy Sampling

Biopsy sampling would be conducted to collect skin, blubber, muscle, or other tissue (see below for details) samples. Sampling may occur on free ranging animals and captured animals during research activities. Only skin and blubber biopsies would be collected remotely during research activities. Skin and blubber biopsy sampling from a vessel may be conducted using crossbows, compound crossbows, dart guns, or pole spears. The depth of the biopsy tip penetration would vary depending on the species being sampled, the need, and the depth of their blubber layer. For small cetaceans, such as bottlenose dolphins, the biopsy tip used to collect blubber for contaminant analysis penetrates to a depth of approximately 1.0-2.5 cm. Shorter tips may be used when only epidermal sampling is required. A crossbow would be used to collect a sample from animals within approximately 5 to 30 m of the bow of the vessel.

Remote biopsy darts may be used to collect skin and blubber biopsy samples from free-swimming cetaceans. This standard technique involves using a blank charge in a modified .22 caliber rifle to propel a dart with small cutting head 3-6 m into the side of a dolphin, below the dorsal fin. A stopper prevents the dart from penetrating to a depth greater than the thickness of the blubber and aids in the removal of the sample form the animal. The floating dart is retrieved, and the approximately 1 cm diameter by 1.5 – 2 cm long sample is processed for archiving and analysis. A video camera mounted on the sampling rifle allows evaluation of the response of the dolphin to the darting.

Pole spears would be used to collect skin and blubber biopsy samples from small, bow-riding cetaceans. The biopsy tip is attached to the pole spear (approximately 5.5 m in length), which is tethered to a vessel. The pole spear is lowered to within 0.5 m of the target, which allows a specific area of the animal to be targeted with a high degree of accuracy.

Blubber biopsies may be taken during health assessment studies. An elliptical wedge biopsy is obtained from each animal. For small cetaceans, the sampling site is located on the left side of the animal, just below the posterior insertion of the dorsal fin. Local anesthetic (typically Lidocaine) is injected in an L-block at the biopsy site. A veterinarian then uses a clean scalpel to obtain a sample that is approximately 5 cm long and 3 cm wide, through nearly the full depth of blubber (approximately 1.5-2.0 cm). A cotton plug soaked with ferric subsulfate is inserted into the site once the sample is removed in order to stop bleeding. The sample is then partitioned into separate containers for each project. Skin obtained with the blubber biopsy is used for genetic analyses. Skin scrapings, biopsy samples, or needle aspirates will be collected for clinical diagnoses from sites of

suspected lesion. These samples are processed by various diagnostic laboratories and a subsample is sent to the National Marine Mammal Tissue Bank.

Biopsy sampling may also occur on animals in rehabilitation for diagnostic purposes. Skin and blubber may be collected as described above for capture animals. Biopsy sampling for diagnostic purposes would also include surgical procedures. Samples may be taken from muscle, lymph nodes, masses, abscesses, liver, kidneys, and other organs. Surgical procedures would be performed by experienced marine mammal veterinarians.

Small muscle biopsies may be collected from pinnipeds. The procedure has been performed on a number of different pinniped species without adverse effects or complications (Kanatous *et al*. 1999; Ponganis et al. 1993). Prior to sampling, a local anesthetic will be injected subcutaneously and intramuscularly at the sampling site to minimize pain. The sampling site will be cleaned with a Betadine scrub and a small incision will be made with a scalpel blade. All biopsies will be taken using appropriately sized sterile biopsy punches at the incision. The punch will be pushed through the blubber and into the muscle layer and the biopsy (~50 mg) is then withdrawn and pressure is applied to the wound. The biopsy site will be irrigated with Betadine. Sutures are not needed for the wound.

1.1.12 Blood Sampling

Blood sampling in cetaceans may be collected from the dorsal fin, caudal peduncle, pectoral flipper, or flukes. Sampling at any of these sites would be done using an 18- gauge 4-cm needle, with a scaled down needle bore for calves, Dall's porpoise, and harbor porpoise. Blood sampling of small cetaceans during health assessments may occur in the water prior to coming aboard the vessel, or once aboard the vessel. Typically, the blood sample is drawn from a blood vessel on the ventral side of the fluke, using an 18-20 gauge ¾" catheter. Approximately 200-350 cubic centimeters (cc) of blood are removed from each individual. The samples are placed in a variety of Vacutainers and other containers specific to the analyses, and are stored in a cooler until they are transported to a laboratory. Some samples may be processed on deck with a portable centrifuge system. Samples are separated and prepared for: standard chemistry, hematology, and hormonal analysis; contaminant analyses; immune function studies; aliquots for culturing for assessment of pathogens; and other preparations as necessary.

Blood samples in both phocids and otariids may be collected through the bilaterally divided extradural vein, which overlies the spinal cord. Otariids may also be sampled using the caudal gluteal

vein. Sampling would be done with a 20-gauge, 4-cm needle for small animals and an 18-gauge, 4-cm needle for larger animals. Phocids may also be sampled by inserting a needle into the metatarsal region of the hind flipper (Geraci and Lounsbury 2005).

1.1.13 Breath Sampling

Breath sampling may be conducted on both ESA-listed and non-listed cetaceans to assess their nutritional status and health for research purposes only. Breath sampling will not be used as a diagnostic tool at this time. A specially designed vacuum cylinder would be used to collect breath samples. The system has previously been used on several cetacean species and elephants. Samples would be collected from free ranging cetaceans by positioning a funnel at the end of a pole (which is connected to the vacuum cylinder via plastic tubing) over the blowhole of the surfacing animal. The cylinder valve would be manually opened during exhalation. An algal culture plate inside the funnel would be used for bacterial cultures of the breath. The culture plate would be sealed and transported to a laboratory for analysis. The equipment typically would not touch the animal, although in some instances there may be brief (less than 10 seconds) contact. An individual animal may be approached up to three times to obtain a sample, if it is exhibiting avoidance behaviors. If an animal exhibits rapid evasion during approaches, the animal will not be pursued. Samples may also be collected during health assessments, emergency response activities, or on any live captured animal. Sampling is being conducted to determine if it may be an appropriate diagnostic tool. Samples will be taken from targeted populations at specific times to compare with visual assessments and/or biopsies. The samples will then be examined using gas chromatography-mass spectrometry for volatile compounds to evaluate respiratory disease, nutritional status, and physical condition.

1.1.14 Ultrasound Sampling

Ultrasound sampling may be conducted on all free ranging animals, animals captured during emergency response, or any species during research studies. Ultrasound may be used to evaluate blubber thickness, wounds, lesions, the presence of lesions, pregnancy, reproductive organs, and blood vessels. Ultrasound may also be used to evaluate cardiac function, other internal organs, and the presence of fat or gas emboli. B-mode, 2-D, and 3-D imaging may be used on marine mammals. Any standard diagnostic ultrasound unit with a “scroll” or “zoom” capability (to visualize deeper structures) would be used to examine marine mammals (Brook *et al.*2001). Transducer type will depend on the area of interest and the size of the patient. Chapter 26 of the *CRC Handbook of Marine Mammal Medicine* will be used as a reference for equipment and methods of ultrasonography for marine mammals (Brook *et al.* 2001). External and internal (transvaginal and transrectal) ultrasound

procedures may be conducted. During transvaginal and transrectal ultrasounds, a well lubricated transducer probe is inserted into the appropriate orifice to the minimum depth required to visualize the structures being observed. The length and diameter of the probe will be determined by the species and individual anatomy. Sedation may be necessary for the comfort of the animal. The level of sedation/restraint is at the discretion of the attending veterinarian. Cetacean ultrasounds will be conducted, as often as possible, while the animal is in water.

For example, during health assessment studies of bottlenose dolphins, a diagnostic ultrasound is used to examine the condition of the internal organ and to measure testis length and diameter to assess male maturity. Females are also examined by a veterinarian during the initial evaluation for pregnancy and the presence of developing follicles. The ultrasound operates at a frequency of about 2.5-5.0 MHz, well above the dolphin's hearing. The examinations are recorded on video and audio tape, and thermal prints are made of features of interest. In addition, digital video thermography is used to measure skin temperature.

1.1.15 Tooth Extraction

The age determination of animals is conducted using the deposition of growth layer groups in teeth. A tooth is extracted from the animal by a veterinarian trained in this procedure. Tooth extraction typically occurs during cetacean health assessment studies. The tissue surrounding the tooth (usually #15 in the lower left jaw of cetaceans) is infiltrated with Lidocaine without epinephrine (or equivalent local anesthetic), applied through a standard, high-pressure, 30 gauge needle dental injection system. Once the area is anesthetized, the tooth is elevated and extracted using dental extraction tools. A cotton plug soaked in Betadine, or equivalent, solution is inserted into the alveolus (pit where the tooth was) as a local antibiotic and to stop bleeding. This plug is removed prior to release. This procedure is modified from that described by Ridgway et al. (1975), wherein the entire mandible was anesthetized. The revised procedure has been used in captivity and in live capture and release sampling for many years. Extracted teeth are sent to a laboratory for age determination.

Tooth extraction in pinnipeds requires capture, restraint, and sedation. In pinnipeds, the post-canine or incisor teeth may be extracted. The tooth and gums are cleaned with an antiseptic solution before, during, and after the tooth is extracted. A scalpel is used to loosen attachments and the tooth is extracted with a dental elevator. Extraction methods would be similar to those described by Arnbom et al. (1992).

1.1.16 Urine Sampling

Urine analyses are diagnostically useful to evaluate the urinary system (kidneys, ureters, bladder, and urethra). Important diagnoses can be made by determining the color, pH, turbidity, chemical constituents, presence or absence of blood, and by identifying any bacteria or yeast present in the urine. These diagnoses would likely be missed without such an examination. Samples may be collected using urinary catheterization. A veterinarian experienced with cetaceans or pinnipeds and a qualified veterinary technician would perform the catheterization procedure. For small cetaceans, the animal would be lying on its side on the foam-covered deck of the boat serving as the veterinary laboratory during health assessment studies. Wearing sterile surgical gloves, the assistant gently retracts the folds of the genital slit to allow visualization of the urethral orifice. The veterinarian (wearing sterile gloves) carefully inserts a sterile urinary catheter, lubricated with sterile lubricating gel, into the bladder via the urethra. A 50 ml collection tube without additive is used to aseptically collect the urine as it flows from the catheter. The catheter is removed after the urine is collected. Pinnipeds would be restrained and sedated before the catheter is inserted. The respiration, heart rate, and temperature of the animal would be monitored during the procedure. The animal would be monitored after the procedure until it is released. Urine may also be collected opportunistically, by holding an open sterile container in the urine stream.

1.1.17 Blowhole Sampling

Microbiological samples may be collected from the blowhole of a cetacean. A sterile swab is inserted into the blowhole during a breath, gently swabbed along the wall of the blowhole, and removed during the next breath. Samples are sent to a laboratory for culturing and species identification.

1.1.18 Fecal Sampling

Fecal samples are obtained either from a small catheter inserted about 10 cm into the colon or from a sterile swab of the rectum. The samples are sent to a diagnostic laboratory for culturing and species identification. Cetacean feces may also be collected in the water column either from a vessel or a diver in the water. Pinniped feces may be collected directly from haul-out or rookery sites. Samples are sent to a laboratory for culturing and species identification.

1.1.19 Milk Sampling

Milk samples are collected to measure the levels of lipophilic organic contaminants and to determine composition. All adult females are checked for lactation and milk samples are collected from all

lactating females. A “breast-pump” apparatus is used to obtain the sample. Milk is expressed with gentle manual pressure exerted on the mammary gland while suction is provided by a 60 cc syringe attached by tubing to another 12 cc syringe placed over the nipple. Samples of up to 30-50 ml may be collected.

1.1.20 Sperm Sampling

A potential impact of environmental contaminants on animal health is the reduction of reproductive capabilities. This may be measured indirectly in males through ultrasonic examination, measurement of testes, and measurement of testosterone concentrations. Collection and examination of sperm samples would be a more direct measurement of male reproductive function. If possible, ejaculate samples would be collected through manual manipulation of the penis. Samples are examined for sperm count, motility, and condition.

1.1.21 Colonic Temperature

Colonic temperature is collected to understand vascular cooling and reproductive status (Rommel *et al.*1992, 1994). Temperature measurements are obtained with a linear array of thermal probes interfaced to a laptop computer. The probes are typically housed in a 3 mm OD flexible plastic tube. The probe is sterilized, lubricated, and then inserted into the colon through the anus to a depth of 0.25-0.40 m, depending on the size of the animal. Temperature is continuously monitored.

1.1.22 Gastric Sampling

Gastric samples may be obtained using a standard stomach tube to evaluate health and evidence of toxin exposure.

1.1.23 Hair, Nails, and Vibrissae Sampling

A vibrissa may be pulled from anesthetized pinnipeds (age limit greater than 2 months). Vibrissae are pulled by gripping with forceps or fingers and pulling forcefully and rapidly in one smooth motion. Nails will be also be clipped close to the base of the nail bed without causing bleeding. Hair samples will be collected with scissors at the base of the hair without removing the follicle.

1.1.24 Administration of Drugs and Euthanasia

Drugs may be administered for sedation/chemical restraint during stranding response and disentanglement activities. These procedures would be performed or directly supervised by qualified

personnel and, if possible, an experienced marine mammal veterinarian would be present to carry out or provide direct on-site supervision of all activities involving the use of anesthesia and sedatives. Anesthetics and analgesics may be used during research before performing biopsies, tooth extractions, and other procedures. Antibiotics, antifungals, and other medicines may be administered during response and rehabilitation of ESA-listed species. Chapter 31 of the *CRC Handbook of Marine Mammal Medicine* will be used as a reference for potential drugs and doses for marine mammal species (Stoskopf *et al.* 2001). Drugs may be administered orally or through injection, intubation, or inhalation. Orally administered medications are typically hidden in fish but may also be given via stomach tube.

Subcutaneous (SQ), IV, IM, intraperitoneal (IP), and intranasal injections may be used to deliver drugs. All of these methods would require some level of animal restraint. SQ injections are made in the interface between the blubber layer and the skeletal muscle layer. Animals must be maintained in a certain position for prolonged periods of time. The most common site for SQ injections in pinnipeds is the craniodorsal thorax between the scapulae. SQ injections would not be used in cetaceans.

In general, IV injections are complicated and rarely used in marine mammals. In cetaceans, medications may be injected in the fluke vessel if the volume is low and the medicine is not harmful if delivered perivascularly. An indwelling catheter may be used if repeated administration or slow infusion occurs (McBain 2001).

IM drug injections require longer needles because of the thickness of skin and blubber. Caution is taken to avoid accidental injection into the blubber, which may cause sterile abscess formation or poor absorption (Gulland *et al.* 2001). Injection into the blubber also has different drug-partitioning properties than muscle. This may result in the failure to activate a systemic distribution of highly lipid soluble medications (Stoskopf *et al.* 2001). Injection sites for phocids are the muscles surrounding the pelvis, femur, and tibia. These sites, as well as the large muscles overlying the scapulae, are appropriate for otariids (Gulland *et al.* 2001). IM injections in cetaceans may be made off the midline, slightly anterior to, parallel to, or just posterior to the dorsal fin. Caution is taken to avoid the thoracic cavity if the injection is anterior to the dorsal fin (McBain 2001). Multiple injection sites may be used and the volume per site should be reasonable depending on the animal.

IP injections deliver medications into the abdominal cavity. Non-irritating drugs may be delivered by this method. During injection, caution must be taken to avoid damaging major organs. A

contaminated needle or puncturing the gastrointestinal tract could introduce bacteria into the abdominal cavity (Gulland *et al.* 2001). Intranasal methods may be used to deliver drugs to cetaceans, via the blowhole (Dunn 2006).

Euthanasia of an ESA-listed animal may be conducted if: an animal had an irreversibly poor condition and rehabilitation would not be possible; rescue would be impossible; or no rehabilitation facility is available. Euthanasia may occur at a rehabilitation facility when an animal is deemed unreleasable and cannot be placed in permanent captivity. Humane euthanasia procedures would only be carried out by an attending, experienced, and licensed veterinarian or other qualified individual. Sedation may precede the administration of euthanasia drugs. Pinnipeds are typically euthanized using a lethal injection of barbiturates or other agent normally used to euthanize domestic species. Smaller cetaceans can be euthanized by injecting barbiturates or other lethal agent into a vein of the flippers, dorsal fin, flukes, or caudal peduncle. It may also be injected directly into the heart of abdominal cavity using an in-dwelling catheter. A small cetacean may be sedated before injection occurred. For large cetaceans, a method is currently being developed to sedate the animal via IM injection and then deliver euthanasia agents via IV. Large cetaceans may be euthanized by lethal injection directly into the heart. Injection into a vein of the flippers or flukes would likely be unsuccessful. Large whales may also be euthanized via intranasal method (injection into the blowhole) (Dunn 2006). Large whales may be euthanized by using ballistics (shooting) or by exsanguination (Geraci and Lounsbury 2005)

1.1.25 Auditory Brainstem Response /Auditory Evoked Potential

Auditory Brainstem Response (ABR) and Auditory Evoked Potential (AEP) procedures may be conducted as a method to evaluate the hearing abilities of individual animals or species. Procedures may be conducted on stranded animals, animals in rehabilitation, or on animals captured during research studies. The ABR technique involves repeatedly playing a test sound stimulus while simultaneously recording the neural evoked potential from surface electrodes.

1.1.25.1 Pinniped Testing Procedures

Pinniped audiometric testing may be conducted while individuals undergo scheduled sedation and/or anesthesia for necessary medical procedures during rehabilitation. SQ electrodes are used for obtaining electrophysiological recordings from pinnipeds and are harmless to the animals. The SQ electrodes are sterile 27 gauge x 10 mm needles that are place subcutaneously beneath the skin on the animals' head. One or two electrodes record AEPs and the other is a reference or ground electrode,

which subtracts the biological noise produced by the animal to enhance the recorded evoked potential responses.

Testing would be conducted under the supervision of the rehabilitation facility's attending veterinarian. Individuals are not tested more than once and testing sessions do not last longer than 60 minutes, except in cases where the individual requires euthanasia upon completion of the anesthetic procedure. Testing time has no impact on animal health or recovery from anesthesia in these individuals. Therefore, in situations where animals require euthanasia upon completion of anesthesia, testing may be allowed to continue for longer intervals at the discretion of the attending veterinarian. This protocol maximizes the amount of information that can be obtained from each subject, improves the quality of the data, and precludes any potential residual impact on anesthetic recovery on the individuals tested. Cases in which animals require euthanasia following anesthesia will be given highest priority in screening for potential study candidates.

1.1.25.2 Odontocete Testing Procedures

Procedures on odontocetes are non-invasive and can be conducted in short time frames. An animal may be resting at the surface or may be physically restrained (held by researchers) during the procedure. ABR signals are collected through suction cup electrodes. Standard EEG gel is used on the electrodes to establish an electrical connection between the electrode and the skin. Sounds may be presented through a jawphone attached to the lower jaw via suction cup. Sounds may also be presented in the water and the animals hear naturally through their lower jaws and other sound paths to the ear. A reference electrode is attached near the dorsal fin and a recording electrode is attached about 5 cm behind the blowhole. The electrodes are on the surface of the skin and are connected to an amplifier via long wires that exceed the length of the tank. The suction cups can easily be removed if there is any difficulty with the procedure. Evoked potentials are recorded from the electrodes. Frequencies used for testing range from 1 to 160 kHz (the range of frequencies that many odontocetes hear) and the maximum sound pressure level is less than 160 decibels re μPa.

Procedures would only be conducted on odontocetes. AEP procedures would not be conducted on mysticetes as there is no documentation on methodology that is likely to be successful in applying audiometric procedures on mysticetes. AEP experiments with animals of this size are inherently difficult for a number of reasons and mysticete anatomy presents additional challenges. All AEP procedures performed on stranded and rehabilitating odontocetes and pinnipeds will follow NMFS PR1 policies and protocols. Testing would not delay treatment, movement, or release of a stranded

animal nor would it interfere with rehabilitation activities. Testing would be stopped if an animal exhibited any adverse reaction, including abnormal respiration and locomotion, vocalization, vomiting, or other signs of distress.

1.1.26 Import and Export of Marine Mammals or Marine Mammal Parts

Exportation privileges are necessary for the MMHSRP to provide specimens to the international scientific community for analyses or as control/standard reference materials and to export animals for release. Importation privileges are necessary for the MMHSRP to acquire legally obtained specimens from outside the U.S. for archival in the National Marine Mammal Tissue Bank or for real time analyses. Importation privileges are also necessary to import live animals for treatment. An unlimited number and kinds of marine mammal specimens, including cell lines, would be imported or exported (worldwide) at any time during the year. Imported and exported specimens would include those taken from the Order Cetacea, Order Pinnipedia (including walrus), Order Sirenia, polar bear, sea otter, and marine otter; this includes threatened and endangered species. Specimen materials may include, but are not necessarily limited to: earplugs, teeth, bone, tympanic bullae, ear ossicles, baleen, eyes, muscle, skin, blubber, internal organs and tissues, reproductive organs, mammary glands, milk or colostrums, serum or plasma, urine, tears, blood or blood cells, cells for culture, bile, fetuses, internal and external parasites, stomach/intestines and their contents, feces, flippers, fins, flukes, head and skull, and whole carcasses. Specimens would be acquired opportunistically; therefore specific numbers and kinds of specimens, the countries of exportation, and the countries of origin cannot be predetermined.

Most specimens would be acquired opportunistically, and the MMHSRP will have minimal control over the age, size, sex, or reproductive condition of any animals that are sampled. However, in cases of prospective or retrospective analyses for a given health related study, these conditions would be provided to NMFS PR1 before activities occur. Imported specimens would be legally obtained from:

- Animals directly taken in fisheries for such animals in countries and situations where such taking is legal and humane;
- Animals killed during subsistence harvest by native communities;
- Animals killed incidental to commercial fishing operations;
- Animals stranded live;
- Animals found dead on the beach or at sea;

- Captive animals, when sampling is beyond the scope of normal husbandry practices or when sampling is taken during normal husbandry practices; and
- Live animals in a permitted, live capture study.

An unlimited number and kinds of marine mammal specimens, including cell lines, would be imported and/or exported (worldwide) at any time during the year. Specimens would be taken from the Order Cetacea and the Order Pinnipedia (except walrus), including threatened and endangered species. Specimen materials may include, but are not limited to: earplugs; teeth; bone; tympanic bullae; ear ossicles; baleen; eyes; muscle; skin; blubber; internal organs and tissues; reproductive organs; mammary glands; milk or colostrums; serum or plasma; urine; tears; blood or blood cells; cells for culture; bile; fetuses; internal and external parasites; stomach and/or intestines and their contents; feces; flippers; fins; flukes; head and skull; and whole carcasses. Specimens are acquired opportunistically; therefore specific numbers and kinds of specimens, the countries of exportation, and the countries of origin cannot be predetermined.

All marine mammals under NMFS jurisdiction, including ESA-listed species, may be imported or exported for medical treatment. Transport methods would be the same as those described in Section 1.1.5.

2. New ESA/MMPA Permit Activities

This Section describes scientific research and enhancement activities that may potentially be conducted under the new ESA/MMPA permit.

2.1.1 Blood Sampling

Currently, no procedures exist to remotely collect blood from free-swimming animals. However, if blood sampling procedures are developed and approved within the timeframe of the permit (five years), the MMHSRP would use these to conduct research. All protocols (including species) would be provided to NMFS PR1 for approval prior to any research activity.

2.1.2 Health Assessment Studies

In addition to the current health assessment studies on bottlenose dolphins, future studies would be conducted on other cetacean species. New tagging, tracking, and telemetry packages would also be used. All species and methods would be provided to NMFS PR1 for approval before any activities occurred.

2.1.3 Acoustics

The use of AEP procedures on any mysticete would not occur under the current ESA/MMPA permit. However, if a successful methodology for applying audiometric procedures on mysticetes is developed within the timeframe of the permit (five years), the MMHSRP would likely use these to conduct research. All protocols (including species) would be provided to NMFS PR1 for approval prior to any research activity.

Passive acoustic recording would involve the used of a hydrophone (underwater microphone). A hydrophone would be placed in the water directly off of a vessel or in a pool, and sounds would be recorded and taped via an apparatus on the vessel or on the pool deck. The purpose of passive acoustic recording is to record the vocalizations of a group of animals and/or the background noise in an area around the group of animals. Passive acoustic recording also indirectly provides background information on noise and vocalizations.

Active acoustic playbacks would be used to expose cetaceans and pinnipeds to playbacks of pre-recorded songs, social sounds, and feeding calls of that species. Playbacks may be used during capture and release activities and during rehabilitation. Sounds and songs would be projected from an underwater speaker hung over the side of a small vessel or in a pool. Sounds or songs would be

projected from the speaker at a volume and quality as close to a real sound/song as possible. The playback system would be calibrated so precise levels of sound can be projected. The physiological and/or physical response of the animals to the sounds and songs would be measured, often through behavioral observation and photographs/video recording of the subject animal(s). Playbacks would be used to determine if an animal can hear and assess how they are responding to sounds. This information would be used to determine the releasability of a rehabilitated animal.

2.1.4 Cognitive Assessment of Sea Lions in Rehabilitation Suffering from Domoic Acid Intoxication.

This study is designed to increase the extent of clinical assessment of California sea lions exposed to domoic acid. Standard veterinary clinical procedures have been used to evaluate the health and prognosis for survival of these cases, including hematology, serum biochemistry, MRI, EEG, and satellite tagging to monitor released animals. Work to date on sea lions (Goldstein et al. 2008) and parallel studies in laboratory animals suggest that there may be additional impacts on sea lion health due to changes in behavior and cognitive function. In an effort to qualify and quantify the cognitive effects of domoic acid exposure on California sea lions, subjects will be assessed will in rehabilitation using behavioral methods. Performance will be evaluated on simple tasks designed to reveal aspects of cognitive function, including auditory habituation, behavioral flexibility, spatial memory, and object recognition. Both passive (observational) and active (food reward) approaches will be used. Direct human contact will be minimized and should not exceed that typically experienced in a rehabilitation setting.

The California sea lion subjects to be assessed will be selected by the veterinary staff at The Marine Mammal Center (TMMC) (Sausalito, CA) from the pool of animals undergoing rehabilitation. Subjects will include prescreened animals identified as domoic acid exposed (by fecal samples, EEG, MRI, and basic neurological assessment) and an equal number of prescreened controls with no apparent neurological deficits (e.g., trauma and malnutrition cases). A maximum of 50 exposed sea lions and 50 controls will be evaluated, but the actual number of subjects will depend on animal availability during the course of the study. Animals of all ages will be examined, based on the availability of stranded animals. Assays will be conducted at TMMC or at the Long Marine Laboratory's (Santa Cruz, CA) marine mammal holding facilities. Each subject will be evaluated during a period not to exceed 30 days. Medical care, feeding schedules, and activity levels for subjects will be similar to those provided for animals in standard rehabilitation settings. Upon completion of their participation, subjects will be assessed for release, continued care, or euthanasia

by the TMMC veterinary staff according to their standard operating procedures. Decisions on the disposition of each animal will be based on medical condition and the ability to survive in the wild, according to the NMFS release guidelines for marine mammals in rehabilitation.

3. References

Arnbom *et al.* 1992 — Arnbom, T.A., N.J. Lunn, I.L. Boyd, and T. Barton. 1992. Aging Live Antarctic Fur Seals and Southern Elephant Seals. *Marine Mammal Science* 8(1): 37-43.

Dierauf 1994 — Dierauf, L.A. 1994. Pinniped forensic, necropsy and tissue collection guide. NOAA Technical Memorandum NMFS-OPR-94-3. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Silver Spring, MD.

Dunn 2006 — Dunn, J.L. 2006. Multiple-agent euthanasia of a juvenile fin whale, *Balaenoptera physalus*. *Marine Mammal Science* 22(4): 1004-1007.

Galimberti *et al.* 2000 — Galimberti, F. and S. Sanvito. 2000. Marking of southern elephant seals with passive integrated transponders. *Marine Mammal Science* 16(2): 500-504.

Geraci and Lounsbury 2005 — Geraci, J.R. and V.J. Lounsbury. 2005. Marine Mammals Ashore: A Field Guide for Strandings, Second Edition. National Aquarium in Baltimore, Baltimore, MD.

Gulland *et al.* 2001 — Gulland, F.M.D., M. Haulena, and L.E. Dierauf. Seals and Sea Lions. In: *CRC Handbook of Marine Mammal Medicine*. Second Edition. L.A. Dierauf and F.M.D. Gulland, eds. CRC Press LLC, Boca Raton, FL.

HSWRI 2006 — Hubbs-SeaWorld Research Institute (HSWRI). 2006. Workshop to Evaluate the Potential for Use of *Morbillivirus* Vaccination in Hawaiian Monk Seals. Final Report. San Diego, CA.

IATA 2006 — International Air Transport Association (IATA). 2006. *Live Animal Regulations.* 33rd Edition. International Air Transport Association, Montreal, Quebec, Canada.

Jeffries *et al.* 1993 — Jeffries, S.J., R.F. Brown, and J.T. Harvey. 1993. Techniques for capturing, handling and marking harbour seals. *Aquatic Mammals* 19.1: 21-25.

Kanatous *et al.* 1999 — Kanatous, S. B., L. V. DiMichele, D. F. Cowan, and R. W. Davis. 1999. High aerobic capacities in the skeletal muscles of pinnipeds: adaptations to diving hypoxia. *Journal of Applied Physiology* 86:1247-1256.

Litzky *et al.* 2001 — Litzky, L.K., R.C. Hobbs, and B.A. Mahoney. 2001. Field report for tagging study of beluga whales in Cook Inlet, Alaska, September 2000. In: Marine Mammal Protection Act and Endangered Species Act Implementation Program 2000. Alaska Fisheries Science Center Processed Report 2001-06. A.L. Lopez and R.P. Angliss, eds. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, Seattle, WA.

McBain 2001 — McBain, J.F. 2001. Cetacean Medicine. In: *CRC Handbook of Marine Mammal Medicine*. Second Edition. L.A. Dierauf and F.M.D. Gulland, eds. CRC Press LLC, Boca Raton, FL.

McLellan *et al.* 2004 — McLellan, W.A, S.A. Rommel, M. Moore, and D. A. Pabst. 2004. Right whale necropsy protocol. Final Report to NMFS for Contract No. 40AANF112525.

Merrick *et al.* 1996 — Merrick, R.L., T.R. Loughlin, and D.G. Calkins. 1996. Hot-Branding: A Technique for Long-term Marking of Pinnipeds. NOAA Technical Memorandum NMFS-AFSC-68. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, Seattle, WA.

Petras 2003 — Petras, E. 2003. A Review of Marine Mammal Deterrents and Their Possible Applications to Limit Killer Whale (*Orcinus orca*) Predation on Steller Sea Lions (*Eumetopias jubatus*). AFSC Processed Report 2003-02. National Ocean and Atmospheric Administration, National Marine Fisheries Service, Seattle, WA.

Pugliares *et al.* 2007 — Pugliares, K.R., A. Bogomolni, K.M. Touhey, S.M. Herzig, C.T. Harry, and M.J. Moore. 2007. Marine Mammal Necropsy: An Introductory Guide for Stranding Responders and Field Biologists. Woods Hole Oceanographic Institution Technical Report WHOI-2007-06. Woods Hole, MA.

Raverty and Gaydos 2004 — Raverty, S.A. and J.K. Gaydos. 2004. Killer whale necropsy and disease testing protocol. SeaDoc Society, Wildlife Health Center, University of California Davis, School of Veterinary Medicine.

Reeves *et al.* 1996 — Reeves, R.R., R.J. Hofman, G.K. Silber, and D. Wilkinson. 1996. Acoustic Deterrence of Harmful Marine Mammal-Fisheries Interactions: Proceedings of a Workshop Held in Seattle, Washington, 20-22 March 1996. NOAA Technical Memorandum NMFS-OPR-10. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Silver Spring, MD.

Ridgway *et al.* 1975 — Ridgway, S.H., R.F. Green, and J.C. Sweeney. 1975. Mandibular anesthesia and tooth extraction in the bottlenose dolphin. *Journal of Wildlife Diseases* 11:415-428.

Rommel *et al.* 1992 — Rommel, S.A., D.A. Pabst, W.A. McLellan, J.G. Mead, and C.W. Potter. 1992. Anatomical evidence for countercurrent heat exchanger associated with dolphin testes. *The Anatomical Record* 232 (1):150-156.

Rommel *et al.* 1994 — Rommel, S.A., D.A. Pabst, W.A. McLellan, T.M. Williams, and W.A. Friedl. 1994. Temperature regulation of the dolphin testes: evidence from colonic temperatures. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 164: 130-134.

Stoskopf *et al.* 2001 — Stoskopf, M.K., S. Willens, and J.F. McBain. 2001. Pharmaceutical and Formularies. In: *CRC Handbook of Marine Mammal Medicine*. Second Edition. L.A. Dierauf and F.M.D. Gulland, eds. CRC Press LLC, Boca Raton, FL.

Thomas *et al.* 1987 — Thomas, J.A., L.H. Cornell, B.E. Joseph, T.D. Williams, and S. Dreischman. 1987. An implanted transponder chip used as a tag for sea otters (*Enhydra lutris*). *Marine Mammal Science* 3(3): 271-274.

USN 2004 — U.S. Navy (USN), Pacific Fleet. 2004. Report on the results of the inquiry into allegations of marine mammal impacts surrounding the use of active sonar by *USS Shoup* (DDG 86) in the Haro Strait on or about 5 May 2003.

Wright *et al.* 1998 — Wright, I.E., S.D. Wright, and J.M. Sweat. 1997. Use of passive integrated transponder (PIT) tags to identify manatees (*Trichechus manatus latirostris*). *Marine Mammal Science*: 14(3): 641-645.

4. Acronyms

ABR	Auditory Brainstem Response
AEP	Auditory Evoked Potential
APHIS	Animal and Plant Health Inspection Service
cc	Cubic centimeter
ESA	Endangered Species Act
HSWRI	Hubbs-SeaWorld Research Institute
IATA	International Air Transport Association
IM	Intramuscular
IP	Intraperitoneal
IV	Intravenous
LHX	Life History transmitter
m	Meter
MMHSRP	Marine Mammal Health and Stranding Response Program
MMPA	Marine Mammal Protection Act
NMFS PR1	National Marine Fisheries Service, Office of Protected Resources, Permits, Conservation and Education Division
NMMTB	National Marine Mammal Tissue Bank
NOAA	National Oceanic and Atmospheric Administration
PIT	Passive Integrated Transponder
SQ	Subcutaneous
TDR	Time-depth Recorder
UME	Unusual Mortality Event
VHF	Very High Frequency

THIS PAGE INTENTIONALLY LEFT BLANK