DRAFT FOR DISCUSSION

The workgroup on animal welfare and ethics has not reached full consensus on the content of this document.

Ethical and Animal Welfare Aspects of Directed Acoustic Research on Marine Mammals

Background and Purpose

During the July 2004, 3rd plenary meeting of the U.S. Marine Mammal Commission Advisory Committee on the Acoustic Impacts on Marine Mammals in San Francisco, the committee formed a workgroup on animal welfare and ethics to address ethical considerations and principles in the context of directed marine mammal research in the wild. The purpose of this document therefore is to provide guiding ethical principles for researchers[1], as well as general examples of experimental designs in the context of the principles that are proposed.

Experiments involving controlled exposures of noise to free ranging marine mammals and of auditory brainstem response in stranded animals have been identified as approaches to filling critical data gaps for understanding effects of noise on marine mammals. However, controlled exposure experiments (CEEs) raise animal welfare concerns because they involve intentional exposure of marine mammals to sound, and the exposure thresholds that may cause pain or stress are not agreed upon.

While support is not universal for the use of CEEs to study acoustic impacts on marine mammals, these experiments are already being conducted in various regions of the world. As a result, stakeholders on all sides have recognized collectively that animal welfare and ethical principles and guidelines must be developed and integrated into CEEs. It is the full intent of this workgroup that this document will be integrated with and strengthen the Synthesis Subcommittee's report, and ultimately help the entire committee make recommendations concerning animal welfare aspects of this research.

Questions and information addressed in this document include:

1) What ethical and animal welfare tenets should be observed in conducting research on animals in the wild?
(2) What are the ethical challenges raised by CEEs?
(3) What is the role of directed research in the wild in the management system for impacts of sound on marine mammals?
(4) Recommendations of the committee on ethical guidelines for Controlled Exposure Experiments (CEEs)? How do the sample guidelines apply to CEEs with beaked whales?
(5) Recommendations of the committee on ethical guidelines for Auditory Brainstem Response (ABR) testing?
(6) [Placeholder for recommendations of the committee on ethical guidelines for temporary capture experiments.]

[1] This document addresses only research conducted in the wild, considering that the preponderance of species of concern are virtually impossible to observe in a captive situation, and ethical protocols and standards already exist for the treatment of captive animals subject to controlled experiments.

To address these questions, this document outlines general ethical and animal welfare principles for field research overall, for specific issues of animal research directed at conservation policy and environmental management, and highlights particular concerns for certain types of experimentation such as CEEs. A specific example is given exploring ethical issues for CEEs with beaked whales.

General Principles for Research on Vertebrate Animals

Nearly all professional societies of scientists conducting research on animals have adopted ethical principles for the treatment of animal subjects in experiments. Most funding agencies require compliance with all applicable laws for animal welfare and conservation. Most scientific journals require authors to state that the research follows such principles. Here, we list some of the ethical principles used in these standards.

Humane treatment of vertebrate animals used in research requires researchers to:

- Comply with all applicable federal, state, and local laws and regulations

- Minimize potential for adverse impact to subjects to the fullest extent possible. Adverse impacts could also include effects on conspecifics other than the subject (e.g. mother-calf separation) and effects on other species, including disruption to ecological relationships.

- Minimize mental, physical, and social stress to subject animals. However, measuring stress in cetaceans is particularly difficult and in its infancy – it is not clear on how cetaceans physically exhibit stress symptoms, in terms of behavioral responses or blood parameters (getting baseline data for their blood chemistry parameters is fraught with difficulties – as they have to be captured first).

- For experiments that may cause mental, physical, social or ecological harm, research must be approved by an appropriate review board using the following criteria:
    - experimental protocol should not be approved if an alternative with less harm is available and equally effective;
    - such harm must be justified by the research proponent or principal investigator as compared to balancing criteria (to be defined), and other important factors. For instance, the probable benefits to science, society or nature should clearly outweigh the foreseeable harms to animals or ecosystems.

- Make a strong commitment to contributing ongoing research to assess long-term impacts to the individual and cumulative impacts to all of the individuals in a population.

- Adhere to the three 'Rs' --reduction, refinement and replacement:
    - ✓ use the fewest number of animals required to demonstrate a specific level of effect. However, the goal should be to try to achieve adequate sample sizes from both sexes to cover the full range of ages and motivational states as is required to demonstrate a

specific level of effect, and to be statistically sound to avoid or minimize ambiguity in results. We recognize that this may not always be possible in field situations.
- ✓ cause the least amount of physical or psychological harm (recognizing that the latter is in most cases very difficult to predict and measure, but should be addressed to the extent practicable).
- ✓ minimize the harassment of animals or populations in research.
- ✓ maximize the use of alternatives to the direct use of animals.

- Require the involvement of highly trained researchers to ensure proper and protective implementation of research techniques. Researchers must assume responsibility for the humane treatment of experimental animals under all circumstances.

- All assistants in the research who might affect the subjects should have received instruction in research methods, and know how to recognize and minimize any adverse impacts of their activities, commensurate with their role in the research team.

- Ensure that the research is not duplicative of previously published work (allowing for replication, which is an essential part of the scientific method).

- Ensure that research results are not misused or misrepresented, for instance by invalid extrapolations, or conclusions that go beyond what the data show, etc.

Research on wild animals for the purposes of management

The research considered in this document is designed to protect wild animals from threats posed by human activities in their natural environment. Most regulations protect by limiting harm or adverse impact, but the threshold exposure for harm is not known for many chemicals or for noise. To protect entire populations of animals in the wild, some believe that it may be necessary to test the effects of exposure that are common in their natural environment. This can lead to a situation where risk for individual subjects is a trade-off to provide benefits to the greater population. Hence, in this context, balancing the risk to the subject(s) against the benefits to entire populations is a key issue.

To this end, applied research that involves the use of sound to understand disturbance responses should focus on providing information needed for management, for which the conservation and/or animal welfare benefits clearly outweigh any potential harm to subject populations. Moreover, applied research that involves the use of sound should also be linked to these management goals, in that it demonstrates its potential contribution to conservation management decision-making. The results should contribute to management programs where they can be applied toward the improvement of the overall well being of individuals, populations, and the ecosystems upon which they depend for survival.

Furthermore, the development of effective policy to protect marine mammals and manage impacts of noise requires risk evaluation. The appropriateness of any particular research method cannot be evaluated in isolation, but must be considered as part of an intermediate stage of

reducing the risks of noise to marine mammals. The first stage involves identifying a hazard, such as epidemiological data suggesting a link between exposure to a pollutant and health problems. The next stage involves two steps that can be taken in parallel: (1) determining the probability of exposure in the population and (2) experiments to define the dose:response relationship between exposure and the hazard. Once these steps have been completed, it is possible to calculate the total effect of the summed exposure to the hazard for the population. A final stage involves comparing the benefits, costs, and other impacts of different strategies to manage the risk.

Scientists conducting experiments on risks to wild animals must be careful to balance the risks to their subjects against the benefit to the study population. Generally, the more endangered a species is, the more precautionary the approach must be in considering research methodologies. For example, there are fewer than 350 right whales in the N. Atlantic, and mortalities continue from fishing gear entanglements and ship collisions to the extent that some models predict extinction. It is particularly important to study the effectiveness of ways to reduce risks to a highly endangered species. Yet no study should be so realistic as to pose the risk of killing or injuring a right whale, since every individual counts for the survival of the population. This situation calls for safe experimental models to approximate the risky situations, or work with surrogate species that could be used to extrapolate to right whales.

Some sound source exposures, such as shipping, seismic, and military sonar, may be common enough to argue for testing individual animals, even at some risk to those individuals, in order to better define any risks to marine mammals, and that mitigation strategies are effective in reducing the risk. In this setting, there must be an honest assessment of risk to the research subject(s), and we recommend that investigators in this situation should have their proposals reviewed by an independent ad hoc committee to judge whether the conservation benefit to the population outweighs the risk to the welfare of the individual subjects.

Specific Principles for Controlled Exposure Experiments (CEEs)

Background

Researchers use a variety of approaches to evaluate the effects of manmade sounds on marine mammals. These techniques include longer-term monitoring of populations and the activities that may affect them, studies involving intermediate spatial and temporal scales, and short-term investigations of individual response. The most effective mix of research approaches will obviously depend on the problem or question being addressed. Our knowledge of marine mammal populations is so poor that we may not be able to detect a decline until it is too late (Taylor et al. 2000), and it may be impossible to identify the causes of the decline. This suggests the need for shorter-term studies on the responses of individual animals to risks such as noise. One approach involves studies that attempt to correlate effects on marine mammals with ongoing sound activities that are not controlled by the investigator. This is one practical way to study large-scale activities, but these studies often lack power to detect all potential effects (e.g. Richardson et al. 1985). [For example, while Rankin concluded that cetacean distributions are related to environmental conditions and not to sound intensity level of seismic exploration at a

large scale, her type of study could not determine any effects for the immediate area of the sound source (Rankin 1999).]

Concerns about limitations in uncontrolled studies on the effects of noise pollution have led to the development of CEEs to study the behavioral responses of a targeted individual whale to directed human-introduced sound (e.g. Malme *et al*., 1984). CEEs investigate effects of noise pollution on individuals by intentionally exposing them to controlled doses of a particular human-made sound. The intention is to accurately record the sound level that is received by the animal at the stage at which a particular behavioral response occurs. A large part of each experiment focuses on the received levels that animals will tolerate before showing 'significant' adverse reactions[2]. By their very nature, these types of experiments may pose a risk to the target animals and the welfare of these animals must be fully considered and protected to the maximum level possible as balanced against the potential gain to the population (as evaluated by an ad hoc committee described above) when considering and/or performing CEEs. In the U.S. these experiments must be authorized with a scientific research permit under the MMPA.

Controlled exposure experiments share some properties with "playback" experiments, a technique that ethologists have long used to investigate animal behavior (especially communication in birds, amphibians and some land mammals). CEEs differ from playbacks in their careful procedures to determine the relationship between measured acoustic exposure and probability of behavioral response. While playback experiments have seldom been controversial, many use sounds of predators or conspecifics that, if successful often evoke strong responses. If CEEs use procedures to slowly increase exposure levels of manmade sounds, they are, in most cases, no more likely to risk strong responses of their subjects than standard playbacks. Another important aspect of CEEs is the control over exposure. Whenever researchers follow marine mammals in a motorized vessel, and especially if the vessel operates depth sounders etc., they unintentionally expose the animals to uncontrolled sound. While intentional exposure may be controversial, carefully controlled exposures can introduce less risk than the uncontrolled exposures (depending on the source) whose risks they are designed to mitigate.

To minimize risks to subjects, the following suggested guiding principles should be incorporated into the design and implementation of CEES:

1) Experiments need to be designed to test hypotheses about specific responses of concern (e.g. disruption of foraging behavior) for particular sources and marine mammals. A well designed experiment can test for a specific dose:response relationship, but cannot rule out all potential effects. In general, the lack of a measurable response to an acoustic stimulus cannot be used to infer that a particular exposure is 'safe' with respect to any and all conceivable risks.

[2] The term 'significance' occurs in more than one country's legislation yet is defined in none. A recent report of the U.S. National Academy of Sciences (NRC 2004), is devoted entirely to the definition of biological significance and proposes a model to predict significance of behavioral disruption induced by noise for individuals and populations.

2) Include 'end-points' for research. End-points define the upper limit of exposure of current management interest and also define where the harms are too great to continue with one or more aspects of a research project or management activity. End-points can also define the lower limit of management interest where the expectation of response is so low (assuming control data exist), that the costs to conduct experiments are difficult to justify. Moreover, identifiable end-points will vary between species and it will be very hard to determine these threshold levels in advance.

3) Where the source level is sufficiently high to present a risk of injury, then sound levels at the subjects must be carefully controlled to minimize the risk. No animal should ever be intentionally exposed to levels in the wild where injury is possible. This requires extrapolation for species whose thresholds for injury are unknown. Also, even where sound exposure is limited to levels not expected to cause injury, it is possible that behavioral reactions might secondarily pose a risk of injury.

4) CEEs should contribute to management programs where the results can be applied toward the improvement of the overall well being of individuals, populations, and the ecosystems upon which they depend for survival. CEEs may comprise just one part of a larger research and management program.

5) Vulnerability of individuals is a combination of their sensitivity and exposure while the threat to the population is also a function of its conservation status. For some of the most endangered species or populations, even the risk of the experiment may be of concern and the use of a proxy species must be considered.

6) CEEs using large powerful sources may ensonify a large area with non-target animals exposed to elevated sound levels. This is of particular concern when available observational methods cannot detect animals out to the range at which they may be affected. Where a powerful novel source is being tested, CEEs themselves should be subjected to the same risk assessment analysis as suggested above. Best available knowledge should be used to generate suitable models. Adopting a precautionary approach, tests should first be carried out within the bounds of these models and the results obtained should be used to modify these limits.

7) NRC (1994) distinguishes between short-term responses, which because of their brevity may be unlikely to have adverse impact, and long-term consequences that due to their extended duration are inherently more worrisome. This suggests the importance of considering the appropriate time scale when planning CEEs. Demonstration of causation between stimulus and response will usually be simpler and quicker with smaller scale CEEs, so when the policy issues focus at larger scales, a combination of small scale and larger scale CEEs may be needed.

8) Researchers undertaking CEEs should collaborate closely and data should be made available to avoid any unnecessary duplication of experiments

9) Research, which is more likely to yield conclusive results with similar or less effort, should be prioritized, in situations where the risk of harm to the animals is similar.

The following procedures are recommended so that CEEs minimize potential risks of harmful exposure:

- ✓ Clear research and management objectives should be identified, with primary concern for the care and well-being of individuals (whilst considering the statistical power of the data being collected).

- ✓ Experiments should start by exposing animals to low received levels and increase levels gradually up to a pre-determined threshold or until a response is observed. (One potential problem with this approach is that it may result in habituation, leading to a bias in the results with higher response thresholds being observed. This can be addressed once a response is observed at a particular level by repeating the experiment with new subjects starting just below this threshold. Another potential problem is that responses may be subtle and therefore overlooked).

- ✓ Experimenters should be aware of the location and behavior of the closest animals during an experiment and minimize the possibility that there are other undetected animals closer than this. If species other than the subjects might be as sensitive, special attention should focus on preventing inadvertent exposure to these species.

- ✓ Work should only be conducted in good conditions of weather and with equipment such that safety of the researchers and research subjects is maximized and the possibility of not detecting animals close to the source will be minimized. The possibility that under these operating conditions animals may be close to the source and not detected should be estimated as part of the risk assessment.

- ✓ Only conduct CEEs when all factors—weather, equipment, team etc.—are of the highest quality and funding is adequate. This will maximize the useful data collected from each exposure and minimize the number of exposures required to answer a particular question.

- ✓ The potential for CEEs to disrupt other scientific research or commercial activities such as whale watching should be considered and minimized. Working in collaboration with teams conducting long-term research programs on populations allows testing for unexpected long term impacts.

- ✓ Within species, studies should be conducted preferentially on populations for which long-term data are already available. Within populations, it would be ideal to study both sexes and all age classes and behavioral states. Given that there are always limitations on meeting this goal, those classes thought likely to be most vulnerable to short-term and long-term disruption (e.g. calves, mothers, breeding animals) should be given highest priority. At first sight CEEs on calves may seem hard to

justify; however, if achievable, it would be more precautionary to extrapolate from more vulnerable individuals to less vulnerable individuals than *vice versa*. Research that does not measure effects on the most vulnerable individuals may seriously underestimate the degree to which they may be affected.

- ✓ Investigators should have their proposals reviewed by an independent ad hoc committee to judge whether the conservation benefit to the population outweighs the risk to the welfare of the individual subjects.

Specific Discussion of Ethical Issues for CEEs to beaked whales

A growing number of fora, including the ACCOBAMS Scientific Committee, European Cetacean Society, and the MMC Beaked Whale Workshop, have identified as a priority the need for research to develop a better understanding of the effects of sonar and other loud sounds on beaked whales. This consensus has stemmed from agreement that analysis of sound fields from the best-studied cases suggested a low probability that the stranded whales could have been exposed to sound levels likely to cause direct injury. Exposure of beaked whales to some threshold of level and duration might trigger a behavior that could lead to a lethal stranding. Since behavior can be triggered at any detectable level, it is critical to measure what exposures start to pose a risk of behavioral disruption. This kind of CEE must be designed to minimize risk to the subject, and this risk must be balanced against the conservation benefits to the population for the potential information derived from the experiment. Given a growing level of support for CEEs to beaked whales, we discuss ethical guidelines here to help the full committee consider the bounds for achieving consensus about such experiments.

The problem of understanding risk factors for beaked whales exposed to mid-frequency sonars illustrates many of the ethical dilemmas raised in this document. As discussed in the report of the Marine Mammal Commission Beaked Whale Workshop, there is a correlation between naval sonar exercises and atypical mass strandings of several species of beaked whales. While we know that beaked whales have stranded within hours of sonar exercises, it is impossible to know what exposures were associated with a risk of stranding because we cannot know where the animals were when they heard the sounds that led to the strandings. Educated guesswork can suggest an exposure range, but these estimates are very uncertain. Some of the stranded whales had injuries consistent with acoustic trauma, or a decompression-like syndrome. It is unknown whether sound from these sonars directly injures animals, whether it triggers a behavioral reaction that secondarily causes injury, or whether injury and death are primarily a result of stranding.

Even though there has been growing interest in the hypothesis that the risk to beaked whales stems initially from a behavioral reaction to sonar, there are alternate hypotheses that beaked whales may have some special vulnerability to sound either to the auditory system or for non-auditory physiological effects. If sound directly causes injury at levels much lower than expected based upon data from other species, and if the injury would not be detected in CEEs, then CEEs would not be appropriate. Risks could be estimated with computer models based upon anatomy from dead animals, but ultimately the only way to test these models directly would be to conduct

controlled tests on hearing or non-auditory effects on the species involved. Such tests would either require innovative methods with wild animals or bringing beaked whales into controlled conditions, probably in captivity. This document suggests a principle that CEEs should not involve exposures that could lead to injury. If we combine this principle with refusal to extrapolate from different species, this would suggest that CEEs to beaked whales should not be conducted until after risks of injury have been quantified. Since beaked whales have never been maintained in captivity for long, this could take decades.

If the risk of stranding is related to exposures that evoke a risky behavioral response, rigid application of our principles for not conducting CEEs until we are certain about levels that cause injury directly could prevent or delay acquisition of data critical for protecting beaked whales from sonar sounds. On the other hand, results from CEEs may not provide information that is conclusive enough to clarify any uncertainty and may result in no additional protection for beaked whales. Any policy to protect beaked whales from sonar will have to rely upon monitoring for whales near planned or ongoing operations. A critical parameter needed to protect these whales is to know the safe exposure zone. This is particularly difficult to estimate until we confirm one of the many hypotheses about the cause of the strandings.

One solution to this dilemma would be to simultaneously move ahead with low level CEEs along with research on risk factors for direct injury. An initial phase of CEEs could use levels below those reasonably expected to pose a risk of direct injury. Such a level could be determined in a planning workshop. The properties of the stimuli used in these initial CEEs should be selected to minimize risk to the subject while maximizing sensitivity for detecting the start of reaction that could, if prolonged pose a risk. We know that beaked whales take many minutes to surface from their deep foraging dives. As long as their behavioral responses are not prolonged well beyond the sound exposure, this would suggest first exploring responses to short sounds starting at low levels up to an endpoint determined by the workshop. If there is great concern that a short stimulus might elicit a prolonged response, this could be tested in an initial phase of the CEE. If a response is seen that might, if prolonged, pose a risk, the CEEs should be halted for that subject. If not, the duration of exposure might be lengthened at the endpoint level. Once a certain number of subjects are tested for onset of risky behavior, the analyses should be publicly reviewed before more exposures are proposed.

Beaked whales are difficult to sight, and methods for passive acoustic monitoring of their sounds are just being developed. This heightens concerns about ensuring that no undetected animals are closer to the source than the subject of the CEE. Not only should this work be conducted under excellent sighting conditions, but ideally should also involve passive acoustic monitoring of the study area in real time. It may be possible to use such passive acoustics to monitor beaked whales during actual sonar exercises, but the risk to the whales may be reduced and the information gained maximized by controlled vs. uncontrolled exposures.

The full committee needs to discuss the optimal mix of monitoring and mitigation measures available right now for beaked whales, along with the balance of research required to understand and reduce the risk of sonars for beaked whales.

Auditory Evoked Potential Hearing Measures in Stranding and Rehabilitation Situations

There is a need to know more about the hearing of marine mammals in general, but urgency is increased because of concerns about auditory damage due to noise pollution. One way that has been recently used to rapidly test hearing is to measure the Auditory Brainstem Response (ABR) of dolphins or whales by passively measuring brainwave patterns from the skin surface. These tests were developed in captive facilities, but there is increasing interest in testing live stranded animals. While this technique provides an obvious value for the determination of hearing abilities of stranded animals and may also expand the knowledge base to include the hearing values of a variety of species that may likely not be kept in captive situations, the use of a new technique calls for ethical guidelines.

ABR is a procedure for measuring hearing through the use of passively measured brainwave patterns. It is routinely used with human infants and it is poses very little additional risk to the animals. It can be used in many situations. This ethical discussion is limited to the situation of measuring ABRs on live stranded or beached marine mammals or stranded and beached marine mammals that have been removed to rehabilitation facilities.

The overriding consideration for dealing with stranded and beached marine mammals is the welfare of the animals. The primary goal in dealing with stranded animals is to assess their condition, determine whether or not they are healthy, and if they are healthy to return them to the wild. If ABR tests of animals that strand and beach are going to be conducted, all concerned, including the scientists, must agree that the primary goal for dealing with any stranded marine mammal is the proper care and welfare of the animal and that these experiments should in no way compromise that care.

There are many considerations to take into account when dealing with stranded animals. Researchers must recognize that the research will not be the number one priority when dealing with stranded animals in a stranding situation. Those responsible for the animal's care will necessarily be in charge of the animal. ABR audiometry provides a diagnostic tool for the veterinarian.

Suggested Guiding Principles:

- Researchers have an obligation to assist in the care of the animal and to do everything possible to assist in the care of the animal.
- Given that the animal's welfare and care is the number one priority, researchers must work closely with the attending veterinarian.
- It is important no animals should be moved into a rehabilitation facility for the sole purpose that ABR measurements can be taken.

The first priority for stranded animals is release if they are judged fit, the second may be to take them to a temporarily to a rehabilition facility until they are fit for release, the third (when an animal is obviously in need of longer term care) is to house them in a rehabilitation facility until they are judged fit and then released with proper follow-up, and finally -only if they are judged non-releaseable- to be properly cared for within a research facility.

- Experienced/trained researchers should use the latest and most up to date equipment to obtain the most data as rapidly as possible. Frequencies should cover a very broad range and amplitudes should start low and ramp up.

- Those conducting research should be very highly trained and proficient prior to attempting this sort of hearing test on a beached or stranded animal.

- Should the animal show obvious increased signs of stress due to sound presentation, tests should be halted.

- If animals are stranded following intensive noise exposure, AEP hearing tests should be used to measure the effects of noise exposure whenever feasible.

- Hearing tests should be used as diagnostic tests. Has the animal been over exposed to sound? What is the current state of the animal's auditory system? Assessment of the animal's auditory system may be useful in deciding whether to immediately attempt release or rehabilitate the animal.

- Researchers must recognize that when dealing with a new species the data may represent the norm for that species or they may represent a damaged animal. The difference between those two cases may only become evident with repeated opportunities to measure the hearing of that species.

- The importance of these hearing measures dictate that they be published rapidly in peer-reviewed literature so that they are available for management and policy decisions, but care should be taken so that these experiments aren't used to prematurely show an 'acceptable' frequency range or level of intensity for a species that is inaccurate and could be used to justify certain noise levels within the cetacean environment that could in fact be damaging to their wild counterparts

[Placeholder for section on Temporary Capture Experiments]

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