13 September 2018

Dr. Nature McGinn, ACA Permit Officer
Permit Office, Room 755
Division of Polar Programs
National Science Foundation
2415 Eisenhower Avenue
Alexandria, Virginia 22314

Ms. Jolie Harrison, Chief
Permits and Conservation Division
Office of Protected Resources
National Marine Fisheries Service
1315 East-West Highway
Silver Spring, MD 20910-3225

Dear Dr. McGinn and Ms. Harrison:

On 11 September 2018 the National Science Foundation (NSF) published a notice (83 Fed. Reg. 46193) requesting comments on a permit amendment application from Dr. Linnea Pearson, California Polytechnic State University. Dr. Pearson is seeking authorization under the Antarctic Conservation Act (the ACA) to amend her ACA permit 2018-013 to conduct research on Weddell seals in Antarctica. The Marine Mammal Commission (the Commission), in consultation with its Committee of Scientific Advisors on Marine Mammals, reviewed the amendment request with regard to the goals, policies, and requirements of the ACA. The Commission also provided extensive comments and recommendations in its enclosed 13 July 2018 letter on Dr. Pearson's amendment request for permit 21006 issued by the National Marine Fisheries Service (NMFS) under the Marine Mammal Protection Act (the MMPA)[1]. Both permits authorize researchers[2] to harass, capture, handle, restrain, measure/weigh, sedate, mark, sample, conduct procedures on, and/or attach instruments to up to 10 Weddell seal pups per year. The purpose of the research is to (1) determine the thermoregulatory strategies by which Weddell seal pups maintain euthermia in air and in water and (2) examine the development of diving capability as the animals prepare for independent foraging.

Public review and comment

Dr. Pearson requested numerous amendments to her ACA permit (see the ACA amendment application for details). Some of the proposed ACA amendments, particularly the ones involving the types of drugs and the ages of pups to which the drugs would be administered, differ from those included in her related MMPA amendment request (see the NMFS amendment application request from June 2018). The Commission recommended that NMFS deny Dr. Pearson's request to amend

[1] The Commission provided extensive comments on Dr. Pearson's original permit application in its 21 July 2017 letter and extensive informal comments on permit application 18879 submitted by Dr. Heather Liwanag, who originally proposed to conduct the activities. Although Dr. Liwanag's application was published for public comment in August 2016, it was subsequently withdrawn.

[2] Researchers also are authorized to conduct ground-based surveys, to collect samples from dead seals, and for up to two pup mortalities per year, not to exceed three mortalities during the permit duration.

her sedation protocol as authorized under the original permit 21006[3]. The Commission found the request to be based on inconsistent and unsubstantiated justifications for using specific drugs for pups 1-week of age and an apparent misunderstanding of what was authorized, thereby resulting in violations of authorized sedation protocols under both the MMPA permit[4] and the research protocols approved by Dr. Pearson's Institutional Animal Care and Use Committee (IACUC). The Commission also found the MMPA permit amendment application to be incomplete and misinformed. The Commission recommended that, before submitting another application seeking to modify her sedation plan, Dr. Pearson (1) review the current NMFS permit and submit an amendment request based on what is currently authorized, (2) discuss with experienced veterinarians appropriate/alternative drug options based on the activities to be conducted and age classes of pups, and (3) stipulate in any amendment request what drugs would be used on the four specific age classes for both Cohort A and B pups, including justification for what drugs are necessary for which activities. The Commission understands that Dr. Pearson submitted revised amendment requests to both NMFS and NSF in August. Although NSF requested comment on the revised ACA amendment request, NMFS apparently has determined it unnecessary to make the revised MMPA amendment request available for public review and comment. The Commission disagrees with NMFS's determination.

NMFS identified Dr. Pearson's original amendment request as a major amendment in accordance with its implementing regulations (50 C.F.R. § 216.39) and thus made it available for public comment in June 2018 (83 Fed. Reg. 30701). Given that any subsequent substantive modification to the proposed sedation protocols would constitute a further change to the manner in which the animals would be taken or otherwise affected and because the proposed change may result in an increased level of take or risk of adverse impacts on the animals, the second amendment request also should be considered a major amendment in accordance with NMFS's implementing regulations at 50 C.F.R § 216.39(a)(1)(ii). As such, the revised amendment request is required to be made available for public comment under 50 C.F.R § 216.39(c)(1). By not doing so, NMFS violated its own regulations that require public notice and comment of all proposed major amendments. Relying on the public notice published in June 2018 and the opportunity for public comment provided at that time is insufficient to meet the requirements of section 104(d)(2) of the MMPA or NMFS's implementing regulations (50 C.F.R. § 216.33(d)), because the public never was given the opportunity to review or comment on the sedation protocols that NMFS is now considering authorizing. Moreover, because the permits issued for these activities under the ACA and the MMPA should be consistent, the agencies do not have the option of issuing one amendment based on NMFS's June 2018 notice and the other based on NSF's notice, which included the updated proposed sedation protocols. The Commission therefore recommends that NMFS and NSF ensure that they are considering the same requested permit amendments and that NMFS suspend further consideration of modifications to Dr. Pearson's sedation protocols considered in the June 2018 notice if they no longer reflect what is being proposed. The Commission further recommends that, if the proposed protocols have changed since they were made available for public review and comment, NMFS publish a new notice in the *Federal Register* and provide a new opportunity for public review and comment prior to considering any such request further.

[3] Which would in turn require denial of Dr. Pearson's request to collect blood samples[3] from sedated pups in Cohort B at 1-, 5-, and 7- to 8-weeks of age.
[4] The ACA permit included the same sedation protocols and thus was violated as well.

ACA amendment request for sedation

As articulated in its enclosed letter, the Commission questions the necessity of using both midazolam and butorphanol to sedate animals in Cohort B[5]. Dr. Pearson originally proposed to use midazolam by itself because it should induce sufficient sedation and muscle relaxation without causing deep sedation or loss of awareness, which will help ensure continued vocalizations with the mother and reduce the chance of maternal abandonment of pups 1-week of age. Specifically, midazolam is a benzodiazepine used routinely in pinnipeds to reduce stress; whereas, butorphanol is an opioid analgesic generally used for short-acting pain relief. Haulena and Schmitt (2018) noted that combinations of benzodiazepines and opioids are used to increase sedation for procedures such as tooth extractions or gastroscopy—both of which are more invasive than collecting morphometric measurements, attaching/removing instruments, and/or collecting blood samples. Dr. Pearson indicated in her ACA amendment request that midazolam and butorphanol is the currently recommended sedative combination for harbor seals based on Haulena and Schmitt (2018). That assertion is not accurate. Haulena and Schmitt (2018) stated that intravenous midazolam and butorphanol followed by masking with isoflurane or sevoflurane is the recommended general *anesthetic* protocol in harbor seals. There are no recommended *sedative* combinations for harbor seals. Rather, Haulena and Schmitt (2018) reported that intravenous diazepam is commonly used alone to *sedate* harbor seals and butorphanol has been used at low intramuscular doses in harbor seals as a *sedative* to aid during restraint. They further indicated that butorphanol at higher doses or butorphanol at low doses in combination with diazepam has been used for endoscopies and obtaining muscle biopsies, procedures again that are more invasive than those to be conducted on Cohort B pups.

Dr. Pearson stipulated that if adequate sedation is not achieved with midazolam alone in the first three 1-week old pups handled[6], then the veterinarian may use midazolam with butorphanol for the remaining 1-week old animals. The Commission notes that each animal reacts to sedatives differently[7] and the procedures to be conducted for Cohorts A and B are not the same. That is, use of sedatives should not be based on another animal's insufficient reaction to them. Moreover, pups in Cohort B are handled and subjected to the metabolic chamber for an hour or two before the researchers attempt to sedate them. Thus, those animals may already be overly agitated and may not react to the sedatives in the same manner as pups in Cohort A that are sedated immediately after being weighed. This also could explain why Dr. Pearson observed apnea in 33 percent of the sedations during the first 10 to 30 minutes after the initial sedatives were given. Of those, 78 percent occurred in animals that were just previously subjected to in-water trials in the metabolic chamber. Breath holding is routine when animals submerge in water, and pups have a greater chance of exhibiting respiratory distress on butorphanol than midazolam or another benzodiazepine. This could be further exacerbated by the animals' behaving as though they are still submerged. The Commission again asserts that it is easier to reverse negative effects from a single family of drugs than two different families of drugs. Therefore, it would have been more prudent to add intravenous diazepam rather than butorphanol to midazolam.

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[5] Only after metabolic measurements have been obtained.

[6] Other portions of the ACA amendment application indicated that the veterinarian could use either midazolam alone or midazolam and butorphanol for 1-week old pups, depending on the animal's activity and at the discretion of the veterinarian.

[7] This also is referenced in Dr. Pearson's sedation summary submitted as part of her annual report under the NMFS permit. One of the pups was more sensitive to sedatives than the other pups and exhibited cyanosis in addition to apnea.

In addition, Dr. Pearson requested to sedate 7- to 8-week old pups in Cohort B with midazolam and ketamine if the animals are refractory to midazolam and butorphanol or were refractory to midazolam and butorphanol at a previous handling[8]. Ketamine is not reversible nor is it necessary for collecting morphometric measurements, removing instruments, and collecting blood samples. As noted during the Commission's informal comments on Dr. Liwanag's withdrawn permit application, ketamine can be taken into the field to have on hand in case of emergencies or medical necessity (i.e., wound debridement) but should not be needed to perform the various activities—activities that at that time were proposed to be more extensive than those currently authorized for Cohort A pups, let alone Cohort B pups. Furthermore, use of a riskier drug combination should not be based on behavior of the animal a few weeks prior, it should be based on whether the animal is sufficiently sedated at the time.

The Commission contends that researchers should be sedating pups only with the types and quantities of drugs that are absolutely necessary to conduct the authorized procedures successfully and safely. It is not convinced that either butorphanol or ketamine is necessary for sedating Weddell seal pups in Cohort B. More importantly, various portions of the ACA amendment application, including the sedation protocol table, are inconsistent and it is unclear exactly what Dr. Pearson is proposing. This has been an ongoing issue with aspects of both Pearson's and Liwanag's original NMFS and ACA permit applications, Pearson's NMFS and ACA amendment applications, and the IACUC protocols[9]. Based on all of these ongoing issues, the Commission recommends that NSF deny Dr. Pearson's request to amend the sedation protocol from what was originally authorized under permit 2018-013[10]. Modifications to the sedation protocols should not be approved until they are internally consistent and NSF has consulted with other veterinary and Weddell seal experts versed in sedation of very young pups regarding the appropriateness of using the various drugs at the multiple ages and for the activities to be conducted on Cohort B pups. Moreover, escalation in the types of drugs used should be based on the behavior of the individual animal at that time, not the behavior of either other animals or the same animal at previous capture times.

The Commission understands that the IACUC protocols also have been revised again. However, it is unclear whether those revised protocols match the procedures stipulated in the ACA amendment application, are internally consistent, and have been approved by the IACUC. All of these must be completed before the 2018 field season.

Sample size sufficiency

The goal of Dr. Pearson's study design was to conduct the various activities on 10 pups in Cohort A and 10 pups in Cohort B. Four pups in each cohort were handled last year, which resulted in Dr. Pearson's request to increase the sample size in 2018 to six pups for each cohort. That request is not unreasonable. However, in reviewing the MMPA and ACA annual reports, it appears that the various procedures were conducted on only a fraction of the animals in 2017. For example, accelerometers were attached to five of eight animals, and three of those accelerometers were lost

[8] Other portions of the ACA amendment application indicated that midazolam and ketamine would be used only for older animals that are very active or agitated, after one of the other sedation protocols has been used.
[9] The Commission also notes that inconsistent numbers of animals were reported to have been taken during the various activities within and between the MMPA and ACA annual reports.
[10] Which would in turn require denial of Dr. Pearson's request to collect blood samples from sedated pups in Cohort B at 1-, 5-, and 7- to 8-weeks of age.

within one week of attachment. Additionally, 4 of 16 possible muscle biopsies were collected. It is unclear whether similar success rates will be achieved this year or whether an increased quantity of data collected this year will make the entire combined dataset large enough to support statistically significant analyses.

More concerning are some of the results from the metabolic trials. In more than 31 percent of the in-air trials, pups became agitated after approximately 35 minutes in the chamber, prematurely ending some trials, and pups exhibited reaming behavior[11] in more than 18 percent of the in-air trials. During in-water trials, pups exhibited reaming behavior in 90 percent of the trials and a pup tried to swim and thrashed around the chamber in 9 percent of the trials, resulting in termination of the trial. Agitation, reaming behavior, and trashing are not conducive for obtaining accurate resting metabolic rate measurements. These shortcomings lead the Commission to question whether the benefits of obtaining the data outweigh the risks to such young pups and whether the *bona fide* requirement would ultimately be met under the MMPA.

Activities previously conducted but not authorized

As stated herein, the researchers, including the veterinarian, did not abide by the underlying NMFS and ACA applications and IACUC protocols regarding what drugs were stipulated to be used on various ages of pups or whether sedation was even authorized to be used on some ages of pups. The Commission understands that the researchers and veterinarian interpreted the NMFS permit conditions to allow for the veterinarian to use any authorized drugs at her discretion. However, it is unclear how the permit conditions could have been interpreted in such a manner, as the only condition remotely close to allowing the veterinarian to use her discretion with authorized drugs involves animals that show signs (e.g., overexertion, constant muscle tensions, abnormal respiration or heart rate) that may lead to serious injury, capture myopathy, other disease conditions, or death. That condition does not, and did not, apply to all animals carte blanche. Dr. Pearson indicated in the ACA amendment application that she was given permission from NMFS to perform sedation on Cohort B pups at 3-, 5-, and 7- to 8-weeks of age. The Commission understands that NMFS attempted to confirm that she was authorized to use sedation on 3-week old animals in Cohort B[12]. However, the researchers interpreted NMFS's response to mean that they could sedate pups in Cohort B at 3-weeks of age, as well as 5- and 7- to 8-weeks of age. Dr. Pearson also indicated in the ACA application that midazolam and butorphanol were used successfully in 2017 to sedate pups from 3 to 7 weeks of age but failed to report that they sedated animals in Cohort A at 1-week of age with those drugs as well.

Dr. Pearson's MMPA and ACA annual reports indicated that VHF tags were attached to two pups in Cohort A and two pups in Cohort B at 3-weeks of age during the 2017 field season. Neither the NMFS nor the ACA permit or the IACUC protocols authorized pups in either cohort to be instrumented with VHF tags at 3-weeks of age. Accelerometers were authorized to be attached at 3-weeks of age and VHF tags at 5-weeks of age. Thus, three of the 3-week old pups were instrumented with both accelerometers and VHF tags attached to the dorsal pelage with epoxy. It is unclear whether Dr. Pearson was aware that the VHF tags were attached to the wrong age class, as

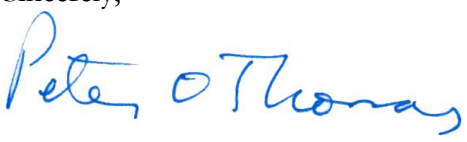
[11] Weddell seals 'ream' the sides of the ice with their canines and incisors to open holes in the ice for breathing and to ensure current breathing holes remain open.
[12] The only age class for which sedation was authorized for Cohort B pups under the NMFS permit.

she indicated in the ACA amendment application that the researchers were authorized to attach those tags at 5-weeks of age. In addition, the annual reports indicated that rectal temperatures were taken on pups of all ages in Cohort B. However, the permit applications indicated that rectal temperatures were to be taken only on pups that participated in in-water metabolic trials in Cohort B and were 3-weeks of age and older.

As detailed herein and in the Commission's July 2018 letter, Dr. Pearson has not complied fully with the requirements of the existing permits. Violations of the MMPA and ACA and any permits issued under those statutes have subjected permit holders to various penalties, including fines, permit revocation, and suspension of the opportunity to obtain new permits. Although the violations of the existing permits by either Dr. Pearson or the veterinarian seem to be unintentional due to a lack of understanding of what the permits do and do not authorize, they were violations nonetheless. Thus, Dr. Pearson, any associated veterinarian, and all other personnel should be advised specifically that they must abide by the procedures and protocols specified in the permit application or any amendment thereto and all permit conditions. Failure to do so will constitute violations of permit 21006 issued under section 104(c) of the MMPA and permit 2018-013 issued under the ACA and may result in permit suspension or revocation and/or other penalties.

Please contact me if you have any questions regarding the Commission's recommendations.

Sincerely,



Peter O. Thomas, Ph.D.,
Executive Director

cc: Dr. Barbara Kohn, Animal and Plant Health Inspection Service

Enclosure

Reference

Haulena, M., and Schmitt, T. 2018. Anesthesia. Pages 567–606. *In* F. Gulland, L. Dierauf, and K. Whitman (eds.). Marine mammal medicine, 3rd edition. CRC Press, Boca Raton, Florida.

13 July 2018

Ms. Jolie Harrison, Chief
Permits and Conservation Division
Office of Protected Resources
National Marine Fisheries Service
1315 East-West Highway
Silver Spring, MD 20910-3225

Re: Permit Amendment Application No. 21006
(Linnea Pearson, Ph.D.,
California Polytechnic State University)

Dear Ms. Harrison:

The Marine Mammal Commission (the Commission), in consultation with its Committee of Scientific Advisors on Marine Mammals, has reviewed the above-referenced permit amendment request with regard to the goals, policies, and requirements of the Marine Mammal Protection Act (the MMPA). Dr. Pearson proposes to amend her permit to conduct research on Weddell seals in Antarctica—permit 21006 expires on 1 October 2020. Researchers are authorized to harass, capture, handle, restrain, measure/weigh, sedate, mark, sample, conduct procedures on, and/or attach instruments to up to 10 Weddell seal pups per year[1]. Researchers also are authorized to conduct ground-based surveys and would collect samples from dead seals. The purpose of the research is to (1) determine the thermoregulatory strategies by which Weddell seal pups maintain euthermia in air and in water and (2) examine the development of diving capability as the animals prepare for independent foraging.

Dr. Pearson has requested numerous amendments to her permit (see the application for details). One of the requested amendments included sedating pups in Cohort B[2] with a combination of midazolam and butorphanol[3] at 1-week of age. During last year's activities, Dr. Pearson reported that some of the 1-week old animals in Cohort B were difficult to handle and became agitated and stressed during the procedures. Dr. Pearson also clarified that the researchers tried using midazolam alone for the first 1-week old pup in Cohort A[4], but the drug didn't result in any level of sedation. Consequently, they then used midazolam and butorphanol for all pups at all sampling time points,

[1] Researchers also are authorized for up to two pup mortalities per year, not to exceed three mortalities during the permit duration.
[2] Including measuring morphometrics including ultrasound, collecting thermal images, conducting in-air metabolic measurements, and attaching a TDR.
[3] Sedation would not be used on animals during metabolic measurements.
[4] Including a full physiology workup administering sedation, measuring morphometrics including ultrasound, collecting thermal images, administering tritiated water and Evan's blue dye, collecting serial blood samples, collecting muscle and blubber biopsies, and attaching a TDR.

including the remaining three 1-week old pups in Cohort A. Permit 21006[5] currently authorizes Dr. Pearson to use only midazolam for 1-week old pups in Cohort A and midazolam and butorphanol only for 3-week old pups in Cohort B[6]. The original research protocols approved by Dr. Pearson's Institutional Care and Use Committee (IACUC) also stipulated only midazolam was to be used for 1-week old pups in Cohort A and midazolam and butorphanol was to be used only for 3-week old pups in Cohort B[5].

The researchers, including the veterinarian, did not abide by the underlying application regarding what drugs were stipulated to be used on various ages of pups or whether sedation was even authorized to be used on some ages of pups. Specifically, sedating 1-week old pups in Cohort A with midazolam and butorphanol and sedating 5-week old and 7- to 8-week old pups in Cohort B was not authorized by the original permit[7]. Dr. Pearson also did not request in the permit amendment application[8] to sedate 1-week old pups in Cohort A and 5-week old and 7- to 8-week old pups in Cohort B with the combination of midazolam and butorphanol. In addition, justification to use midazolam and butorphanol in 1-week old pups in Cohort B, which was the only request in the amendment application, was inconsistent and unsubstantiated. In one place, sedation was needed to facilitate collecting morphometrics, in another it was to collect blood, and in yet another it was to attach instruments. Compliance and consistency issues aside, there are some underlying questions regarding the necessity of using both midazolam and butorphanol.

Dr. Pearson originally proposed to use midazolam by itself because it should induce sufficient sedation and muscle relaxation without causing deep sedation or loss of awareness, which will help ensure continued vocalizations with the mother to reduce the chance of maternal abandonment for pups 1-week of age. In an earlier version of the amendment application, Dr. Pearson noted that midazolam by itself is sufficient for conducting procedures that last 30 to 40 minutes. Midazolam also is used routinely in pinnipeds to reduce stress; whereas, butorphanol is an opioid analgesic used for short-acting pain relief. The procedures that are authorized to be conducted on pups in Cohort B are much less extensive and invasive than those in Cohort A. In addition, animals have a greater chance of exhibiting respiratory distress on butorphanol than midazolam or another benzodiazepine. Although the drugs can be reversed, such young pups are more prone to possible negative effects than older pups, juveniles, or adults. Further, it is easier to reverse negative effects from a single family of drugs than two different families of drugs. Thus, it may have been more prudent to propose to add intravenous diazepam, which is commonly used in phocids, rather than butorphanol to midazolam. The Commission contends that researchers should be sedating pups only with the types and quantities of drugs absolutely necessary to conduct the authorized procedures successfully and safely and is not convinced butorphanol is necessary for pups in Cohort B. Based on all these issues, the Commission recommends that NMFS deny Dr. Pearson's request to amend the sedation protocol from what was originally authorized under permit 21006, which would in turn require denial of Dr. Pearson's request to collect blood samples[9] from

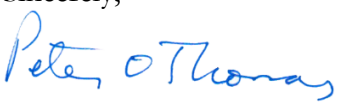
[5] See the original application and longitudinal sampling scheme document.
[6] Permit 21006 does not authorize sedation for 1-week old, 5-week old, or 7 to 8-week old pups in Cohort B.
[7] Or approved by the IACUC.
[8] Or accurately clarify the drug combinations to be used at the various age classes in the revised research protocols that were submitted and approved by her IACUC. The revised research protocols are internally inconsistent, with the original sedation plan in one portion of the protocols and the plan to use midazolam and butorphanol at all four ages classes for both Cohorts A and B in another portion.
[9] Based on requesting to collect blood samples only under sedation.

sedated pups in Cohort B at 1-, 5-, and 7- to 8-weeks of age.

Furthermore, the Commission recommends that NMFS advise Dr. Pearson that, prior to submitting another amendment application for changes to her sedation plan, she (1) review the current permit and submit an amendment request based on what is currently authorized, (2) discuss with experienced veterinarians appropriate/alternative drug options based on the activities to be conducted and age classes of pups, and (3) stipulate in any amendment request what drugs are to be used at the four specific age classes for both Cohort A and B pups, including justification for what drugs are necessary for which activities. The Commission additionally recommends that NMFS advise Dr. Pearson that all research protocols reviewed and approved by her IACUC must match those procedures authorized under her research permit prior to conducting any procedures. Thus, if Dr. Pearson plans to submit another amendment application, she must revise her research protocols yet again, ensure the protocols are internally consistent, submit those revisions to her IACUC for review, and obtain approval before the upcoming field season. Dr. Pearson and all affiliated personnel must abide by the procedures specified in the permit application or amendment application, otherwise they again will be in violation of permit 21006 as issued under section 104(c) of the MMPA.

Please contact me if you have any questions regarding the Commission's recommendations.

Sincerely,



Peter O. Thomas, Ph.D.,
Executive Director

cc: Dr. Barbara Kohn, Animal and Plant Health Inspection Service
Dr. Nature McGinn, National Science Foundation