Animal and Plant
Health Inspection
Service

4700 River Road
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United States Department of Agriculture

July 7, 2023

[redacted].
Eastern Virginia Medical School
Institutional Animal Care and Use Committee Administration, EVMS Research
[redacted]
Norfolk, Virginia 23507

Dear Ms. [redacted]

Thank you for the questions submitted on June 2, 2023, to ensure EVMS's compliance with the Animal Welfare Act and Regulations. Please find responses to each question below.

First, regarding Dr. Pepe's protocol and our review of the protocol during the inspection, our inspectors thoroughly read Dr. Pepe's protocol multiple times. If the protocol is too complex to be understood by trained and experienced veterinary medical officers and agency specialists, as you suggest, it is likely that the protocol is not understood by the IACUC's non-affiliated member, or that it does not clearly describe all the procedures that an animal will experience. In either case, such a protocol cannot be effectively reviewed and approved, as required by the regulations.

Splitting Dr. Pepe's current protocol so that each protocol form describes a single research activity/study may facilitate a more effective and understandable IACUC review and approval. It does not, however, resolve the issue regarding multiple major operative procedures (MMOP). As discussed below, MMOP may be scientifically justified as part of a single animal activity/study, for veterinary care, or under special circumstances approved by APHIS.

Question 1. The MMOP exception was withdrawn based on the observations of the inspectors, including the agency's nonhuman primate specialist. Those observations will not change, regardless of the outcome of the appeals process. The agency takes its obligation to safeguard animal welfare seriously, and MMOP exceptions require careful consideration. When they are granted, it is incumbent on the investigator and institution to ensure that all conditions are met, since animals that undergo MMOP are at increased risk of experiencing pain and distress.

In this case, the inspectors observed unapproved protocol deviations, which violated the first condition in the approval letter. Additionally, EVMS told APHIS that "any animal that shows signs of any pain, distress, or disease gets a clinical evaluation and appropriate treatment as designed by our AV. Such an animal would not be allowed to breed until completely healed, and if not healthy/fully healed, is excluded from the protocol by the AV" in the application for the exception. As another condition of the approval, the IACUC was required to evaluate animal well-being along with the

effectiveness and soundness of methods and procedures. Our inspectors found evidence that neither effective AV nor IACUC evaluation was occurring. For example, animal #07105 lost 18% of her body weight after her first pregnancy and caesarean section, but the weight loss was never addressed. The animal had a second pregnancy while still underweight, and subsequently died from aspiration immediately following the second caesarean section. Other occasions on which uterine abnormalities and/or post-operative clinical issues were noted without documentation of complete resolution as determined by the attending veterinarian were also identified. Accordingly, APHIS had no choice but to withdraw its approval of the exception.

Question 2. APHIS amended the Animal Welfare Regulations to replace the requirement for annual continuing reviews with a requirement of a complete resubmission and review at least every 3 years, effective December 2021. The agency explained in the preamble to the Final Rule that the intent of the 3-year evaluation is for "resubmission and complete review of that activity every 3 years thereafter as if it were a new activity." The Final Rule goes on to explain that this change addresses the situation that you are describing, in which an animal research activity "can continue indefinitely without ever being fully revisited to ensure its underlying design or foundational assumptions are in step with current science and regulatory policy relating to animal welfare." You can find the Final Rule here: https://www.federalregister.gov/documents/2021/11/24/2021-25614/awa-research-facility-registration-updates-reviews-and-reports.

Regarding the 3-year de novo reviews, each MMOP exception is linked to a specific protocol for a specific time frame and, therefore, does not carry over without APHIS approval at the time an old protocol expires and a new one is approved. This is specifically stated in the approval letters we issue.

As you are aware, §2.31(d)(1)(x) requires that no animal be used in more than 1 major operative procedure from which it recovers unless A) justified for scientific reasons in writing by the principal investigator; B) required as routine veterinary procedure or to protect the health or well-being of the animal as determined by the attending veterinarian; or, C) when approved by the APHIS administrator based on other special circumstances.

Additionally, using a single protocol form to describe different research activities does not make them a single study, even if the activities have the same overarching objective (e.g., understanding the role of estrogen in pregnancy). Accordingly, this approach does not alleviate the regulatory requirement that no animal be used in more than one MMOP unless one of the three scenarios listed in §2.31(d)(1)(x) applies.

In order for §2.31(d)(1)(x)(A) to apply, i.e., for there to be an adequate scientific justification for performing more than one major operative procedure on the same animal, the MMOP must be part of the same research activity/study. This is because if each major operative procedure is done for a different research activity/study, then there is no scientific link between the procedures to justify performing MMOP on the same animal. The original issue in this case that necessitated the MMOP exception was documented in the facility's September 2021 inspection report. At that time, the facility was cited for performing MMOP without a scientific justification. Although the protocol described that MMOP are necessary to use the animals as their own controls within a treatment group at different time points, the inspectors found that the same animal had received different treatments, so that the MMOP, in fact, occurred as part of different research activities and were not consistent with the justification provided in the protocol. Thus, APHIS approval of the MMOP was required as a special circumstance.

Question 3. In the Animal Welfare Act, Congress explicitly prohibited using an animal in more than one major operative experiment except in cases of scientific necessity or other special circumstances. The intent of this prohibition is to avoid placing an excessive burden on individual animals. The IACUC should take a balanced approach to considering the 3Rs – reducing animal numbers by performing multiple procedures that cause pain and distress on the same animal is not consistent with the ethical intent of the 3Rs "to promote the humanest possible treatment of experimental animals" (Russell and Burch).

APHIS has not stipulated a specific maximum number of cesarean sections. The IACUC may determine the appropriate number that maintains animal health and welfare. The IACUC and investigator are encouraged to consult the relevant literature related to complications associated with increasing numbers of C-sections and to benchmark with other facilities performing similar procedures.

Question 4. The maximum number of C-sections should be determined by the IACUC. Applying appropriate safeguards that are clearly described in the protocol to maintain animal health and welfare (e.g., routine reproductive examinations via ultrasound by trained veterinary staff; identification of clear, objective criteria for when animals will be excluded due to complications or reproductive abnormalities and who makes this decision; adequate pain management consistent with the current standard of care, etc.) is critical in making this determination. The IACUC may wish to benchmark with other institutions performing similar procedures to determine best practices.

Question 5. As above. §2.31(d)(1)(x) requires that no animal be used in more than 1 major operative procedure unless A) justified in writing by the principal investigator; B) required as routine veterinary procedure or to protect the health or well-being of the animal as determined by the attending veterinarian; *or* C) when approved by the APHIS administrator based on other special circumstances. Provided there is scientific justification in the protocol and approved by the IACUC for multiple operative procedures in the same animal as part of the same research activity, future animals would not need an exception approved by the agency. The research activities must be consistent with the scientific justification provided. In other words, if the investigator justifies MMOP as required because an individual animal will serve as its own control within a single treatment group, the activities performed must mirror that description.

Thank you again for working collaboratively with us to ensure EVMS is compliant with the Animal Welfare Act and Regulations. If you have any additional questions, please contact your inspector, or send inquiries to animalcare@usda.gov.

Sincerely,

Roxanne Mullaney, DVM
Acting Deputy Administrator