DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
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Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

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April 22, 2021

Re: OLAW Case A3352 D

Heather Bragg, MSL
Interim Executive Director
Institutional Official
Carnegie-Mellon University
5000 Forbes Avenue, (b) (4) Building
Pittsburgh, PA 15213

Dear Ms. Bragg,

Thank you for the thoughtful response to our request for information regarding animal welfare concerns at Carnegie-Mellon University dated November 13, 2019, and the follow-up conference call on April 21, 2021. Based on the information provided, our office understands that:

1. Your institution has policies and protections in place regarding animal welfare concerns. Mechanisms for reporting concerns are widely posted, there are provisions to allow anonymous reporting, and the institution has a whistleblower protection policy.
2. There is a post approval monitoring (PAM) program administered by the institution's Office of Researeh Integrity and Compliance. At least 2 protocols per year are subject to PAM review under this program, and the results of each visit are reported to the Institutional Animal Care and Use Committee (IACUC).
3. The institution has an IACUC-approved euthanasia standard operating procedure (SOP), step by step instructions on rodent euthanasia, a requirement for a secondary euthanasia method to confirm death after carbon dioxide euthanasia, and instructional signage at each euthanasia station.
4. Regarding the specific animal welfare concerns involving carbon dioxide euthanasia in the Barth and Gittis laboratories, the IACUC determined that these concerns were valid. Many issues appeared to stem from improper communication. Specifically, in person training for laboratory members did not follow the guidance in the IACUC approved SOP, the IACUC-approved protocols involving euthanasia did not provide procedural details and did not reference the SOP, and animal welfare concerns were not brought to the attention of the IACUC (they were reported to other institutional staff instead). In addition. at least one euthanasia station was not functioning properly. During the IACUC investigation, other animal welfare concerns were discovered, including failure to employ aseptic surgical technique, failure to administer treatments prescribed by the institution's veterinarian, improper post-surgical monitoring, and improper recordkeeping. There was no evidence found to support the allegation that the laboratories handle and dispose of carcasses inappropriately.
5. The institution addressed the issues by retraining the Gittis and Barth laboratory members, and going forward, requiring similar hands on training for all laboratories. Barth and Gittis laboratory members were not permitted to perform unsupervised euthanasia until the Attending Veterinarian or vivarium staff confirmed that their technique is satisfactory. In addition, a novel "train the trainer" approach has been initiated to improve euthanasia technique across all laboratories. The faulty euthanasia station was repaired, all euthanasia equipment was checked for functionality, and all stations are to be checked on an enhanced schedule going forward. Protocol modifications outlining the euthanasia procedure in detail were submitted by the principal investigators for the Gittis and Barth laboratories. Both laboratories were subject to increased PAM oversight and the most recent PAM visits found no

noncompliances. Laboratory members were also required to participate in additional surgical training, an updated IACUC approved surgical SOP with improved guidelines was sent to all laboratories, and there will be increased PAM in relation to surgical activities across all laboratories. Signage for reporting concerns was evaluated to ensure it was up to date and retraining on reporting concerns was conducted. Standard operating procedures are also being developed for recordkeeping and reporting animal health and welfare concerns, which will be distributed to all laboratories once approved by the IACUC. In addition, the animal care and use program is being restructured to improve management.

Based on the information provided, OLAW finds that your institution has been responsive to the concerns outlined in our request for information. OLAW concurs with the actions taken by your institution to comply with the PHS Policy on Humane Care and Use of Laboratory Animals. We commend your institution for continuing to evaluate and improve your compliance program in the spirit of self-regulation, and at this time find no cause for further action by this Office.

Sincerely,

Nicolette A. Petervary -S

Digitally signed by Nicolette A. Petervary -S
Date: 2021.04.22 15:55:09 -04'00'

Nicolette Petervary, VMD, DACAW
Animal Welfare Program Specialist
Office of Laboratory Animal Welfare

CC: IACUC Contact

Carnegie Mellon University

Institutional Animal Care and Use Committee
5000 Forbes Avenue
(b) (4) Building
Pittsburgh, PA 15213

December 22, 2019

Confidential & Proprietary – FOIA Exempt

VIA Email

Nicolette Petervary, VMD, DACAW
Animal Welfare Policy Specialist
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, MD 20892
olawdco@mail.nih.gov
cc: nicolette.petervary@nih.gov

Dear Dr. Petervary,

This letter is to provide you with the requested information regarding the Institutional Animal Care and Use Committee (IACUC) review of the animal welfare related concerns outlined in the Office of Animal Welfare (OLAW) Case ID A3352-01 sent to Carnegie Mellon University (CMU) via a letter dated November 13, 2019 (the "OLAW Letter"). Key events relevant to this review are summarized in Exhibit A.

1. Policies and Protections Regarding Reported Animal Welfare Concerns

a. The IACUC is responsible for investigating all reported concerns regarding the care and treatment of animals used in research or teaching at CMU. Mechanisms for reporting concerns are prominently posted in all individual labs and on the IACUC website (see https://www.cmu.edu/research-compliance/animal-research/report-concern.html). Contact information for the IACUC Chair, Institutional Official (IO), and IACUC Director are provided as well as a hotline number for anonymous reports. In accordance with CMU's Policy Against Retaliation (currently available at https://www.cmu.edu/policies/administrative-and-governance/whistleblower.html), any individual who makes a good faith report of a suspected violation is protected from retaliation.

b. The IACUC's process for handling reported animal welfare concerns is as follows. Upon receipt of a report of an animal welfare concern, the IACUC and IO would promptly assemble an internal committee to conduct an inquiry into the matter, involving CMU legal counsel as needed. The

specific steps taken during such inquiry would depend on the nature of the reported concern and could include, among other things, an inspection of the relevant facility or lab space, interviews of the relevant personnel, and inspection of records. Upon completion of the inquiry, a summary of the findings would be provided to the employee(s) that reported the concern, if known. A report outlining the concern, findings, and any corrective actions taken would also be provided to the IO, and, if appropriate to OLAW.

2. **Post Approval Monitoring**

   a. The Post-Approval Monitor, a member of the Office of Research Integrity and Compliance ("ORIC") staff, conducts post-Approval Monitoring (PAM). The Post-Approval Monitor's role is to observe the research activity, to assist the principal investigator (PI) in identifying any deviations from the approved protocol, to implement any required changes to an approved protocol, and to document the findings of the PAM visit. A PAM visit is expected to be conducted on at least one (1) protocol during any given six (6) month period (e.g. the Post-Approval Monitor is expected to complete at least two (2) PAM visits each year). The Post-Approval Monitor typically uses their discretion to determine which research activity on which protocol to conduct a PAM visit. The results of each PAM visit are reported to the IACUC.

   b. During a PAM visit, the various areas of a research, teaching, or testing project (i.e., the protocol, personnel, study procedures, anesthesia, surgery, post-surgical care, euthanasia, and general lab/record keeping) are all subject to review. Questions included in the PAM Checklist, attached as Exhibit B, can help PIs evaluate their own research, teaching, and testing programs and identify potential noncompliance issues before they become serious and/or reportable problems. The IACUC discusses the completed PAM Checklist, and provides any recommendations to the investigators.

3. **Educational Signage; IACUC Approved Euthanasia Standard Operating Procedure**

   a. On or about October 2018, signage was posted on the walls near each of the three carbon dioxide (CO2) euthanasia stations (222A, 228A, and 224D) in the Mellon Institute Centralized Vivarium (MICV). These stations included those used by the Barth and Gittis laboratories. The signage, which has been continuously present since that date, stipulated procedures consistent with those subsequently embodied in a formal standard operating procedure (SOP), including the appropriate number of rodents for a cage, CO2 flow rates and secondary methods of euthanasia. A copy of the signage is included as Exhibit C to this letter.

   b. The CO2 euthanasia SOP, approved by the IACUC on February 13, 2019, is attached as Exhibit D to this letter. The IACUC considered the SOP to be a facility SOP as it only pertained to the MICV and not the animal program as a whole. The IACUC did not discuss or specify at such time the mechanism through which the SOP would be disseminated.[1]

[1] The IACUC, at its next meeting, will discuss the process by which SOPs will be disseminated.

4. **IACUC Interactions and Issues with the Laboratories of Dr. Alison Barth and Dr. Aryn Gittis**

a. The laboratories of Dr. Alison Barth (Barth) and Dr. Aryn Gittis (Gittis) house animals and conduct some procedures, including CO2 euthanasia, in the MICV. The MICV personnel currently include a Facility Manager and five CMU staff members. The MICV Facility Manager and staff coordinate and interact with the CMU Attending Veterinarian (AV) as needed. Both the MICV Facility Manager and the CMU AV are third party contractors from [redacted] (b) (4)

b. CMU employs laboratory technicians for each of the Barth and Gittis laboratories (although it is our understanding that the laboratory technician for the Barth laboratory also performs certain limited duties for the Gittis laboratory). The laboratory technicians are not part of the MICV staff.

c. In June 2018, the IACUC learned of concerns regarding overcrowding in rodent caging by multiple investigators, including Drs. Barth and Gittis, within the MICV. However, the overcrowding was not in the context of euthanasia procedures involving CO2. A copy of CMU's self-report to OLAW dated July 5, 2018 as well the response from OLAW dated July 9, 2018 regarding this matter are enclosed as Exhibits E and F, respectively.

d. At the time CMU received the OLAW Letter, the IACUC had not received any reports of concerns regarding CO2 euthanasia procedures for either the Barth or Gittis laboratories.

e. In response to the OLAW letter, the IACUC convened an internal committee on November 15, 2019, to look into the animal welfare concerns, and requested the CMU AV discreetly observe and report on the euthanasia practices of one or both labs the following week.

f. On November 18, 2019, the CMU AV notified the internal committee and the MICV Facility Manger that she had witnessed certain CO2 euthanasia procedures underway as she was walking through the MICV that were being undertaken by the Barth laboratory technician. She indicated there appeared to be 18 mice in a single cage and a flow rate that appeared to be too high (estimated 8 liters/min) and a few of the mice seemed to be showing signs of distress (frantic running up the side and rapid pawing in the air). The CMU AV indicated that she then saw the cage after it had been removed from the gas, and that several of the mice appeared to be convulsing and the technician was returning the cage to the gas for an additional period of time. The CMU AV indicated she later returned to the lab and did a postmortem carcass inspection, where she noticed that several animals were visibly bleeding from the eyes and mouth (indicating elevated conscious CO2 levels). The CMU AV made it clear that she only witnessed certain portions of the procedure, and not the entire procedure. So, for example, she could not confirm whether or not a secondary method of euthanasia was utilized since she was not present in the room at such time, and she was unable to tell from the postmortem inspection whether cervical dislocation had been used since the mice had been in the freezer.

g. On November 18, 2019, the MICV Facility Manager (who was not yet aware of CMU's receipt of the OLAW Letter) notified the IACUC of a third reported concern[2] (i.e. the November 18, 2019, notification from the CMU AV referenced above) regarding the performance of CO2 euthanasia (specifically relating to overcrowding and CO2 flow rates) and suggested mandatory training regarding CO2 euthanasia procedures.

h. On November 19, 2019, I sent an email to Drs. Barth and Gittis stating that, until further notice, all CO2 euthanasia had to be performed by MICV staff personnel, and any other form of euthanasia had to be supervised by either the MICV Facility Manager or the CMU AV unless they agreed that a procedure could be conducted without their supervision. The email also instructed Drs. Barth and Gittis to ensure that their personnel attend the upcoming euthanasia training, which by that time had already been scheduled for November 21, 2019.

5. Inquiry

The internal committee reviewed relevant information in the possession of the IACUC and, on December 2, 2019, conducted interviews of relevant personnel including Drs. Barth and Gittis, the two laboratory technicians tasked with carrying out CO2 euthanasia for their laboratories, the MICV Facility Manager and two MICV staff personnel. Outlined below is a summary of the findings:

a. Drs. Barth and Gittis indicated that they do not personally carry out euthanasia procedures, and are not typically in the laboratory during such procedures. They indicated that technicians associated with their laboratories perform CO2 euthanasia, as do, also, certain additional personnel in the Barth laboratory, including graduate students and post docs. Drs. Barth and Gittis indicated that they were not aware of the approved CO2 euthanasia SOP.

b. At the time of the interview, the laboratory technicians associated with the Barth and Gittis laboratories had already taken the mandatory training offered on November 21, 2019, and were aware of the procedures stipulated by the CO2 euthanasia SOP. In addition, in accordance with the mandatory IACUC training requirement for personnel to be to be included on a protocol, the laboratory technicians previously completed CITI training, including the module "Working with Mice in Research." Among other things, this CITI module outlines the optimal flow rate that should be used for CO2 euthanasia. However, the laboratory technicians indicated that they had previously been unaware of the SOP and that they had not noticed the posted signs and/or did not understand that the signs reflected an IACUC-approved SOP or a mandatory set of procedures. They further indicated that their prior in-person training had not been consistent with the SOP. They indicated that they were trained by other laboratory teehnicians (some of whom are no longer at the institution). The in-person instruction they said they received was apparently incomplete and/or outdated in some regards (in particular, not reflecting their CITI training or the post-2013 guidelines of the American Veterinary Medical Association (AVMA). For example, they indicated that the in-person instruction they received did not include information on the maximum number of mice per enclosure or the specific

[2] The MICV Facility Manager's November 18, 2019 correspondence referenced it being the third instance of which she was aware, but at the time the IACUC had not yet been informed of the prior two instances to which she was referring. The IACUC subsequently learned of the prior two instances, which are noted below in the summary of our inquiry findings.

CO2 flow rates to be used. Both technicians indicated that they took steps to confirm death following CO2 euthanasia (such as checking for respiration and/or a heartbeat). However, until their recent in-person training, it was not their understanding that a secondary method of euthanasia was required pursuant to the SOP. With respect to disposal, the laboratory technicians said that they did not personally recall seeing any mouse carcasses left out in the laboratory or not properly disposed of.

c. The procedures carried out by the laboratory technicians associated with the Barth and Gittis laboratories were technically consistent with brief descriptions in the laboratories' IACUC-approved protocols. However, the descriptions in the protocols were not detailed and did not refer to the SOP.

d. During our interview, the MICV Facility Manager indicated that two MICV staff members had reported concerns to her on October 30 and November 8, 2019, regarding CO2 euthanasia-related situations they had observed in the Barth and Gittis laboratories. During a call from the CMU AV to the MICV Facility Manager on October 30, 2019, regarding an unrelated topic, the MICV Facility Manager mentioned the CO2 euthanasia concerns to the CMU AV and discussed setting up a training session on CO2 euthanasia to address the concerns. These MICV staff reports were not provided to the IACUC at such time. The CMU AV was on scheduled time off[3] from October 30, 2019, through November 11, 2019 (including the time such reports were received by the MICV Facility Manager and mentioned in the phone call with the CMU AV). After the CMU AV returned, the training was arranged and scheduled to take place November 21, 2019.

e. The MICV Facility Manager indicated that on or about mid-November 2019, she noticed that mice seemed to be taking too long to become unconscious when she used the CO2 station in 228A. One of the laboratory technicians and one of the MICV staff also indicated that they noticed a similar issue around the same time. Once the MICV Facility Manager became aware of the issue, the euthanasia lid, the CO2 tanks, and the CO2 lines were inspected and ruled out as the cause of the issue. So, although the flow meter being used in 228A was only about a year old, the MICV Facility Manager arranged to have the flow meter replaced for the station in 228A. No further issues regarding flow meter functionality were reported until the CMU AV was notified on December 20, 2019, by a technician in the Barth laboratory that the euthanasia station in 228A did not appear to be functioning appropriately. The CMU AV promptly responded that day to remove the euthanasia cage lid and to hang up two signs (one directly on the CO2 flow meter knob and one on the line that normally connects to the cage lid) indicating that the station was out of order and not to use, and instead to use the station in 222A to conduct CO2 euthanasia. The CMU AV verbally notified the Barth laboratory technician that the 222A station was to be used instead until further notice. The CMU AV also notified the MICV Facility Manager, who is currently out on vacation, of the issue via email. We will keep the 228A station out of commission until the station can be tested to determine if there is an issue (and if so, to remedy the issue). We are not in a position to judge how much, if at all, flow meter malfunction contributed to the reported issues.

f. The two MICV staff members who had reported concerns regarding CO2 euthanasia to the MICV Facility Manager could not confirm that the instances they witnessed occurred on November 2 and 6 (the specific dates noted in the OLAW Letter). However, they did witness instances of deviation from the CO2 euthanasia SOP by laboratory technicians associated with the Barth and Gittis laboratories

[3] CMU arranged for back-up veterinary coverage during such time.

around that time (e.g. instances of overcrowding, no secondary method of euthanasia being used, and improper disposal). For example, one of the MICV staff indicated that she witnessed overcrowding during CO2 euthanasia and no secondary method of euthanasia being used. In another instance, as another MICV staff member was preparing to leave for the evening, the staff member relayed that she entered the Barth laboratory and discovered a large number (approximately 35) of deceased mice left out in a cage on a table. The staff member indicated that the mice were intact, and the MICV staff member felt their necks and could tell that no cervical dislocation had been performed. The MICV staff member notified the MICV Facility Manager, who asked that the MICV staff member perform a secondary method of euthanasia on the mice and place them in the freezer prior to leaving for the evening. In addition to observing incidents that are consistent with the concerns raised in the OLAW Letter, the MICV staff members we interviewed also reported observing the laboratory technicians for the Barth and Gittis laboratories using an initial rate of CO2 flow that was higher than prescribed in the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition and that they observed bleeding from the eyes and/or mouth in certain of the mice (signs of elevated conscious CO2 levels).

g. In the course of our inquiry, we became aware of certain other animal welfare concerns regarding Barth and Gittis laboratories that are unrelated to CO2 euthanasia:

   i. The MICV Facility Manager indicated that on more than one occasion she observed the laboratory technician in the Gittis laboratory failing to use all appropriate personal protective equipment during surgery. The MICV Facility Manager indicated she had spoken to the laboratory technician about appropriate personal protective equipment and subsequently informed the PI. These instances were not previously reported to the IACUC.

   ii. During the timeframe in which the IACUC's inquiry was underway, the CMU AV became aware of three concerns and reported them to the committee. First, the CMU AV noted that she witnessed nonsterile materials being prepared and provided for use in surgery. Second, the CMU AV noticed multiple instances where it was not evident that a treatment prescribed by the CMU AV for an animal in the Gittis laboratory was actually carried out as directed (in one instance, since she indicated that there was not a record of the treatment having been performed and the MICV Facility Manager did not have a record of the applicable medicine being picked up). Third, the CMU AV noted that the laboratory technician in the Gittis laboratory stated to her that she was not aware she needed to record treatments despite written and verbal directions to do so. In addition, both the MICV Facility Manager and CMU AV indicated concerns that treatments for animals were not being documented in a timely manner as required.

   iii. Previously, the CMU AV reported[4] that post-surgical animals belonging to the Barth laboratory were left overnight without being attended to despite multiple emails and in-person conversations between the CMU AV and the laboratory technician throughout the day.

[4] The CMU AV mentioned this incident during the course of our inquiry. However, in following up with the CMU AV for additional information, the committee discovered that this incident had been reported by the CMU AV to various persons, including the IACUC Chair and IO, on May 9, 2019. However, the IACUC was unable to locate records to substantiate that the IACUC's processes for handling reported animal welfare concerns were followed in this instance.

The CMU AV also notified the PI of the incident. Additionally, the CMU AV previously reported[5] an instance of an inappropriate therapeutic treatment being given by a laboratory technician in the Barth laboratory without veterinary authorization and without prior protocol approval. This treatment resulted in euthanasia of the animal.

6. Corrective Action Plan

Upon completion of its inquiry, the IACUC has developed the following corrective action plan to ensure proper training and compliance with its stated euthanasia SOP and to address the other animal welfare concerns and procedural issues described in this letter:

a. A group training session in the MICV, led by the MICV Facility manager with the AV present, was held on November 21, 2019. Such training was mandatory for the laboratory personnel of Drs. Barth and Gittis. Additionally, the MICV Facility Manager has conducted subsequent hands-on training sessions.

b. The IACUC and the MICV Facility Manager are in agreement that euthanasia training should be mandatory for all laboratories. The IACUC will work with the MICV Facility Manager to plan and implement such training sessions. More generally, the IACUC will review current practice with regard to training in other procedures related to animal research in the MICV.

c. As noted above, effective November 19, 2019, the IACUC placed limitations on the ability to carry out CO2 euthanasia in the Barth and Gittis laboratories. The IACUC will permit the individuals in such laboratories to conduct unsupervised CO2 euthanasia only after such individual has received training by MICV staff or the CMU AV and has performed at least one supervised CO2 euthanasia procedure conducted in a satisfactory manner (as determined in the discretion of the supervising individual). The supervision and confirmation of a satisfactory procedure is currently being done by the CMU AV.

d. The MICV Facility Manager has been reminded to report any animal welfare concerns directly to the IACUC.

e. The IACUC has disseminated the approved euthanasia SOP and communicated to all MICV laboratories that they must perform euthanasia in this manner. As part of its review, in the future, the IACUC members will be instructed to ensure that euthanasia methods outlined in protocols are reflective of the approved SOP prior to approval. Accordingly, Drs. Barth and Gittis, as well as the other MICV researchers, shall submit modifications to their current protocols to clarify their specific euthanasia procedures accordingly. In addition, as a general matter, the IACUC will revise the format of its approval letters to clarify that the approval of the protocol is subject to the relevant regulations, policies and procedures (including SOPs).

[5] The CMU AV mentioned this incident during the course of our inquiry. The CMU AV noted that she previously reported the incident to the MICV Facility Manager and to a representative from the IACUC office during a meeting on September 17, 2019. The CMU AV also indicated that she had forwarded certain correspondence about the incident to the same representative from the IACUC office on September 17, 2019. The committee could not locate any reports and/or evidence of escalation to the IACUC Chair or the IO at such time regarding this incident.

f. A PAM visit to each of the Barth and Gittis labs will be conducted prior to next semi-annual inspection in April, 2020.

g. As noted above, the CO2 station in 228A will be checked to determine if it is currently functioning appropriately (and if not, will not be available for use unless and until any issues are remedied). In addition, the functionality of the CO2 equipment shall be checked as a part of the semi-annual laboratory inspection process.

h. Regarding surgical procedures, the IACUC previously disseminated a surgical guideline. A copy of the surgical guideline is attached as Exhibit G to this letter. The IACUC resent the surgical guidelines to all laboratories, will mandate surgical training for individuals in the Barth and Gittis laboratories, and will conduct spot checks of the other laboratories with respect to surgical procedures. The IACUC is in the process of developing additional SOPs to cover appropriate surgery procedures and aseptic techniques, and will offer hands-on training to supplement these materials.

i. With respect to animal health treatment, the IACUC is in the process of developing a SOP for reporting and managing animal health concerns, which will include recording and compliance obligations. The IACUC will circulate such SOP to the laboratories once it is finalized and approved.

j. As noted above, through this inquiry process, the IACUC learned that certain reported animal welfare concerns were not addressed in accordance with the IACUC's processes. The IACUC takes its obligations seriously. The IACUC will review the mechanisms through which animal welfare concerns are reported. In addition, the IACUC will review its current procedures for responding to reported concerns and for appropriately documenting such responses. Following such reviews, the IACUC will revise and/or supplement its existing practices and procedures as necessary so that reports are received by and/or escalated to the appropriate persons for attention by the IACUC and so that such reports are properly addressed in a timely manner.

CMU is committed to the continued growth of our animal program. A meeting with CMU senior leadership is scheduled for early January 2020 to discuss how to best support the needs of the program.

Please let me know if you have any questions, or require any additional information to conclude your review.

Sincerely,

DocuSigned by:
(b) (6)
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Heather Bragg
Interim Executive Director
Office of Research Integrity and Compliance
Institutional Official

cc: Carl Olson, PhD
Carnegie Mellon University
IACUC Chair

(b) (6)

Enclosure

Exhibit A

Timeline of Key Events

October, 2018: Signage stipulating proper CO2 euthanasia procedure posted at all three stations.

February 13, 2019: Rodent Euthanasia SOP consistent with signage approved by IACUC.

October 30, 2019: First report (by MICV staff) to MICV Facility Manager regarding deviation from SOP.

November 2, 2019: Approximate date noted in the OLAW letter of first specific incident.

November 6, 2019: Approximate date noted in the OLAW letter of second specific incident.

November 8, 2019: Second report (by MICV staff) to MICV Facility Manager regarding deviation from SOP.

November 13, 2019: Letter from OLAW notifying IACUC of animal welfare concern.

November 15, 2019: Internal committee formed by IACUC.

November 18, 2019: CMU AV visits laboratory and observes activity deviating from SOP.

CMU AV separately notifies internal committee and MICV Facility Manager of the observed activity. CMU AV's notification to MICV Facility Manager was the third report to the MICV Facility Manager regarding deviation from the SOP.

MICV Facility Manager notifies IACUC of the third report.

November 19, 2019: Barth and Gittis laboratories barred until further notice from performing CO2 euthanasia

November 21, 2019: CO2 euthanasia training session conducted (mandatory for Barth and Gittis laboratories).

December 2, 2019: Committee interviews the PIs, their laboratory technicians, the MICV Facility Manager and MICV staff who had previously reported deviations from the SOP.

December 16, 2019: Certain personnel from Barth and Gittis laboratories cleared, after appropriate retraining and satisfactory performance of the procedures under the supervision of the CMU AV, to resume CO2 euthanasia.

Post-Approval Monitoring (PAM) Checklist Policy for Post-Approval Monitoring Program

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Investigator: _____

Protocol Number: _____

Protocol Title: _____

Species: _____

Date of Monitoring: _____

Procedure Observed: _____

PAM Team Member(s): _____

Date expires: _____

The Protocol and Personnel

Y N N/A 1. Confirm that the PI and research personnel know how to access the most recent version of the complete protocol, including amendments.

Y N N/A 2. Confirm that study team members have read the protocol.

Y N N/A 3. Confirm that laboratory staff performing the procedure(s) listed on the protocol.

Y N N/A 4. Confirm that all personnel currently up to date on Occupational Health Program requirements.

Y N N/A 5. Confirm that each room where animals are taken listed on the protocol.

Study Procedures

Y N N/A 6. Does the protocol number on the animals' cage card match the IACUC approved protocol number?

Y N N/A 7. Are the procedures performed consistent with those approved in the protocol?

Y N N/A 8. Are research personnel appropriately trained to perform these procedures and is documentation of training available?

Y N N/A 9. Are investigators/research personnel wearing appropriate Personal Protective Equipment (PPE) and/or other attire (i.e., gloves, masks, etc.) for the species and procedures performed?

Y N N/A 10. Are the species, strains, and ages of animals consistent with those in the approved protocol?

Y N N/A 11. Are the methods of anesthesia in compliance with the protocol?

Y N N/A 12. Are anesthetized animals monitored according to the approved methods in the protocol?

Y N N/A 13. Are the animals maintained at an appropriate depth of anesthesia for the procedure performed?

Y N N/A 14. If inhalant anesthetics are used, are they scavenged appropriately?

Y N N/A 15. Are analgesic dosages, frequency, and routes of administration accurately recorded?

Surgery

Y N N/A 16. Is surgery performed in a location that has been approved by the IACUC? Is there a separate animal preparation and surgical space?

Y N N/A 17. Is the method of animal prep appropriate and in accordance with the approved protocol?

Y N N/A 18. Is survival surgery performed using sterile instruments, sterile gloves, a surgery mask and aseptic technique?

Y N N/A 19. Is an appropriate heat source used to keep the animal warm throughout the surgical procedure?

Y N N/A 20. Are incisions closed appropriately and in accordance with the approved protocol?

Y N N/A 21. Is there an appropriate/designated recovery area for the animals?

Y N N/A 22. Is there only one major surgery performed on each animal (unless prior approval by the IACUC)?

Y N N/A 23. Is an identification method in place to indicate which animals have had a procedure performed on them?

Post-Surgical Care

Y N N/A 24. Is the post-surgical area in compliance with the approved protocol?

Y N N/A 25. Are the methods of analgesia (dose, frequency, duration) consistent with the approved protocol?

Y N N/A 26. Is post-surgical/post-procedural care adequately documented? Is an appropriate heat source used for recovery?

Y N N/A 27. Are any post-operative problems reported to CMU animal care or veterinarian?

Record Keeping

Y N N/A 28. Is there an up-to-date and complete surgical/procedure log (i.e., USDA medical record, pink card)? Is the animal's weight recorded at appropriate intervals?

Y N N/A 29. Are individual animals appropriately identified (cage cards, ear tags, punches, tattoos, etc.)?

Y N N/A 30. Are medical and post-procedural care progress notes complete and accurate?

Y N N/A 31. Is medication/anesthetic/analgesic administration accurately documented?

Y N N/A 32. Are injections, blood collection, and fluid collection amounts dated and documented?

Euthanasia

Y N N/A 33. Does the method of euthanasia correspond with what is written in the protocol?

Y N N/A 34. Is death assured by performing an approved physical/secondary method of euthanasia?

Laboratory

Y N N/A 35. If USDA species are housed in the lab for greater than 12 hours (24 hours for rats and mice), has the lab been approved for this activity by the IACUC?

Y N N/A 36. Are drugs, suture materials, and other items within their expiration date?

Y N N/A 37. Are controlled substances stored/logged appropriately?

Y N N/A 38. If applicable, are sharps containers located within the lab?

Y N N/A 39. Are there any safety issues or other concerns that pose a threat to human or animal safety, or animal welfare?

Comments/ Clarifications: _____

_____

_____

_____

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_____

Flow meter instructions for euthanasia of Mice and Rats

1. Euthanize animals in their home cages if at all possible and *no more than 5 animals per cage.*
2. Take the top and wire lid off the cage
3. Place the stainless steel euthanasia lid on the cage so that all three holes are over the opening of the cage
4. Turn C02 on at the valve on the wall
5. Adjust the flow meter to the correct rate based on 10%-30% chamber volume per minute in order to optimize reduction of stress. **Let the C02 run for about 5 minutes**.

Cage Size (inches)	Flow Rate (liters/min)
IVC Mouse Cage (32.5cm x 16.5cm x 12.65cm)	1.4
IVC Rat Cage (39cm x 29cm x 19cm)	4.4

6. After the animals become unconscious, the flow rate can be increased to minimize the time of death
7. Shut off CO2 by turning the flow meter knob gently clockwise until the ball inside of flow meter falls to the bottom. **Do not over tighten.**
8. Turn off house CO2 by turning the handle towards the back wall.
9. Verify death by performing cervical dislocation, bilateral thoracotomy, or decapitation. This is per the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

Carnegie Mellon University
Mellon Institute Center
Vivarium
Standard Operating Procedure

1.0 Purpose

This document describes the acceptable methods of euthanasia for species housed at Mellon Institute animal care facility. Euthanasia is performed on animals to alleviate pain, requested by veterinarian due to health concerns, for diagnostic testing, or for research protocols which are approved by the IACUC.

2.0 Responsibilities and Scope

Euthanasia is to be performed by a trained PI or animal care staff according to the 2013 Guidelines set forth by the American Veterinary Medical Association (AVMA). Any modification of this policy must be approved by the IACUC.

3.0 Definitions

Euthanasia – from the Greek meaning "good death", or one that occurs with minimal pain and distress.

4.0 Accepted Forms of Euthanasia

4.1 Primary Form of Euthanasia

4.1.1 Carbon **Dioxide Asphyxiation**

4.1.1.1 This is the most common method of euthanizing mice and rats. This method can also be used on rabbits (under 3kg) and other small mammals. Secondary verifiable method for death must be performed (e.g. bilateral thoracotomy, cervical dislocation, or deeapitation).

4.1.2 Secondary Forms of Euthanasia

4.1.2.1 **Decapitation and Cervical Dislocation**
Pre-anesthetized: Adult rats and mice that are anesthetized may be decapitated or cervically dislocated by a trained individual only. This training must be given by documented trained PI staff or the Facility Manager.
No pre-anestbetic: IACUC approval only
Explicit scientific justification of this method on non-anaesthetized adult rats and mice must be provided and approved by the IACUC. This procedure can only be performed by documented trained PI and animal care staff only.

4.1.3 **Barbiturate overdose** – This method of euthanasia can be performed on rodents but the animal **must be sedated first**. The recommended dosage for euthanasia is found in the product label information. Because these are controlled substances, they must be procured with a DEA license. These preparations can be administered intravenously. **Do not administer these compounds via subcutaneous, intramuscular, or intraperitoneal methods.**

4.1.4 Other forms of euthanasia not discussed in this SOP must be properly described and submitted to the IACUC for review and approval.

5.0 **CO2 Euthanasia—Use of the Low Flow Meter**

5.1 The 2013 AVMA Euthanasia Guidelines require that rodents being euthanized by CO_2 must be subjected to a controlled slow flow of gas for the most humane death. The flow rates are based on the size of the cage and are given below in the table. **Do not pre-charge the chamber!**

5.2 Animal care staff inspect CO_2 tanks daily and replace any tanks which are low.

5.3 Place euthanasia lid on top of animal's home cage making sure that the two open holes on the lid are located within the open area of the cage.

5.4 Turn the flow meter knob counterclockwise gently until the ball reaches the correct flow rate for the size of the cage. **(Please see attached Flow Meter Instructions)**

Cage Size (inches)	Flow Rate (liters/min)
IVC Mouse Cage (32.5cm x 16.5cm x 12.65cm)	1.4
IVC Rat Cage (39cm x 29cm x 19cm)	4.4

5.5 Run CO_2 at the 10–30% flow rate until the animal has lost consciousness. Then turn the gas up to minimize time of death.

5.6 Shut off CO_2 by turning the flow meter knob gently clockwise until the ball inside of flow meter falls to the bottom. Do not over tighten and then turn off house CO_2 by turning the handle towards the back wall.

5.7 Verify death by performing cervical dislocation, bilateral thoracotomy, or decapitation.

5.8 Pups (21 days and younger) are resistant to euthanasia by CO_2 due to their inherent resistance to hypoxia and may require prolonged exposure time to any type of inhalant. Consequently, CO_2 alone should not be used as a sole means of euthanatizing pups. CO_2 may be used to induce narcosis but must be followed with another acceptable method of euthanasia (e.g., decapitation or thoracotomy) to ensure death.

6.0 Training

6.1 Training is required to use CO_2 euthanasia. Contact the Facility Manager to schedule training. No PI or animal care staff may perform CO_2 euthanasia without proper training.

7.0 References

7.1 American Veterinary Medical Association. (2013). *AVMA Guidelines for the Euthanasia of Animals* http://www.avma.org

8.0 Approvals

This document requires the following approvals.

Name	Title
Carl Olson, PhD	**IACUC Chair**

Name	Title
Sara Andux, PhD, DVM, DACLAM	**Attending Veterinarian**

Name	Title
Heather Bragg	**Institutional Official**

9.0 Document History

Revision Number	Revision Date	Summary of Changes	Author

Carbon Dioxide Euthanasia Flow Meter Instructions: Rats

NOTES:

- All rodents should be euthanized in their home cage if at all possible.
- Species should not be mixed
- Should occur in a procedure room or laboratory, away from other rodent housing.

This table serves as a general guide:

RODENT	AGE	MICE[1]	RATS[2]
		Minimum time in 100% CO_2	
Non-haired pups	0 to 6 days	60 minutes	40 minutes
Haired pups, eyes closed	7 to 13 days	20 minutes	20 minutes
Haired pups, eyes open, pre-weaning	14 to 20 days	10 minutes	10 minutes
Weanlings and adults	21+ days	5 minutes	5 minutes

1 Pritchett et al. 2005

2 Pritchett Corning 2009

Steps

- Turn tank all the way on
- Adjust flow meter to 4.40 L / min (for 20% flow rate)
- Allow minimum time as indicated on chart, plus an extra three (3) minutes

- Perform a confirmatory method of euthanasia to ensure death (exsanguination, decapitation, cervical dislocation or bilateral thoracotomy).
- Turn off flow meter and CO_2 tank.

Carbon Dioxide Euthanasia Flow Meter Instructions-Mouse

NOTES:

- All rodents should be euthanized in their home cage if at all possible.
- Species should not be mixed
- Should occur in a procedure room or laboratory, away from other rodent housing.

This table serves as a general guide:

RODENT	AGE	MICE[1]	RATS[2]
		Minimum time in 100% CO_2	
Non-haired pups	0 to 6 days	60 minutes	40 minutes
Haired pups, eyes closed	7 to 13 days	20 minutes	20 minutes
Haired pups, eyes open, pre-weaning	14 to 20 days	10 minutes	10 minutes
Weanlings and adults	21+ days	5 minutes	5 minutes

1 Pritchett et al. 2005

2 Pritchett Corning 2009

Steps

- Turn tank all the way on
- Adjust flow meter to:
  - 1.4 L/min for 20% flow rate
  - 2.03 L/min for 30% flow rate
- Allow minimum time as indicated on chart, plus an extra three (3) minutes
- Perform a confirmatory method of euthanasia to ensure death (exsanguination, decapitation, cervical dislocation or bilateral thoracotomy).
- Turn off flow meter and CO_2 tank.

July 5, 2018

Axel Wolff, DVM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
Rockledge 1, Suite 360
6705 Rockledge Drive
Bethesda, MD 20892

E-mail: olawdco@mail.nih.gov

Dear Dr. Wolff,

Pursuant to PHS Policy, IV.F.3, I'm writing on behalf of Carnegie Mellon University (A3352-01) to follow-up on a preliminary report of a non-compliance. As you and I discussed by phone on June 19, 2018, I was recently advised about overcrowding of rodent cages in our vivarium. I believe this occurred due to a transition in staff and was a limited, short term problem that did not cause any harm to the animals. On June 18, 2018, all principal investigators and animal care staff using the vivarium were contacted about the need to assess their cages and make any necessary adjustments. This has been completed.

New standard operating procedures (SOPs) have been established and communicated to all users of the facility regarding housing and care of mice and rats as well as cage overcrowding due to breeding. These SOPs clarify our standards, how to maintain the standards and how to communicate if concerns arise.

I hope you will accept this as CMU's assurance that we're dedicated to excellent care of animals in our facility and continually improve our programs. Please let me know if you have any questions.

Best Regards,

(b) (6)

Ann G. Mathias
AVP Research Compliance
Institutional Official

Exhibit F

DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY
Office of Laboratory Animal Welfare
Rockledge One, Suite 360
6705 Rockledge Drive – MSC 7982
Bethesda, Maryland 20892-7982
Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

FOR EXPRESS MAIL:
Office of Laboratory Animal Welfare
Rockledge One, Suite 360
6705 Rockledge Drive
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301) 402-7065

July 9, 2018

Re: Animal Welfare Assurance
A3352-01 [OLAW Case C]

Ms. Ann Mathias
Research Integrity Officer
Carnegie Mellon University
5000 Forbes Avenue - (b) (4)
Pittsburgh, PA 15213-3890

Dear Ms. Mathias,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your July 5, 2018 letter reporting a serious deviation from the provisions of the *Guide for the Care and Use of Laboratory Animals* at Carnegie Mellon University, following up on an initial telephone report on June 19, 2018. According to the information provided, OLAW understands that because of a staff transition, several cages of mice were overcrowded for several days. There were no adverse effects reported on the animals.

The corrective actions consisted of counseling the investigators and animal care staff and separating the mice. Standard operating procedures addressing rodent care and appropriate cage densities were established and staff was trained on these. The SOPs also describe how to report any concerns regarding cage overcrowding.

Based on its assessment of this explanation, OLAW understands that measures have been implemented to correct and prevent recurrence of this problem. OLAW concurs with the actions taken by the institution to comply with the PHS Policy on Humane Care and Use of Laboratory Animals.

Sincerely,

(b) (6)

Axel Wolff, M.S., D.V.M.
Deputy Director
Office of Laboratory Animal Welfare

cc: IACUC Chair

Carnegie Mellon University
Mellon Institute
Centralized Vivarium
Rodent Surgery
Guidance Document

1.0 Purpose:

Post-operative infections in rodents can and do occur. To reduce the occurrence of infections which can change the physiological parameters and affect the results of a study, these guidelines need to be followed for successful rodent surgeries.

2.0 Scope:

This guidance document applies to all surgical procedures performed on animals in the MICV facility in which animals are expected to recover from anesthesia.

3.0 Definitions:

3.1 Survival Surgery – Operative procedure from which the animal is allowed to recover.

3.2 Minor Survival Surgery – Any procedure which does not expose a body cavity and causes little or no physical impairment. Minor procedures require aseptic technique, sterile instruments, and appropriate anesthesia.

3.3 Major Survival Surgery – This type of surgery includes entry into the cranial, abdominal or thoracic cavities. This would be any procedure that could render the rodent physically handicapped, either permanently or partially, would be considered a major surgical procedure. Aseptic technique is mandatory in these types of procedures to minimize post-surgical infection.

3.4 Analgesics – A compound capable of producing analgesia, i.e., one that relieves pain by altering perception of nociceptive stimuli (caused by or responding to a painful stimulus) without producing anesthesia or loss of consciousness characterized by reduced response to painful stimuli.

3.5 Impervious materials – Incapable of being penetrated or being affected

3.6 Aseptic technique – Using methods to protect against infection by pathogenic microorganisms

4.0 Procedure:

4.1 Procedure Location:

4.1.1 A separate surgical facility is not necessary for rodent surgery. However, rodent surgeries should be conducted in low traffic, clean areas which promote asepsis during surgery. Procedure rooms are available for research staff to use for surgical procedures.

4.1.2 Surgical tables and equipment must be made of impervious materials that can be disinfected. Equipment made from cardboard and wood are not acceptable.

4.1.3 A surgical suite is available in the MICV facility for research staff to use. Contact the Facility Manager or Animal Care Staff for proper usage procedures of this area.

4.2 Animal Preparation:

4.2.1 Sterile instruments, sterile surgical gloves, and aseptic preparation of the surgical site are necessary to prevent post- operative infection.

4.2.2 The animal must have the hair removed from the surgical site.

4.2.3 Prepare the surgical site with three repetitions of Betadine or Nolvasan solution alternating with 70% isopropyl alcohol. Apply solutions/ alcohol in a circular motion starting in the center of the incision site working your way outwards. Be very careful not to wet down the animal as this could lead to problems such as hypothermia and possible death.

4.2.4 To prevent the cornea from drying out during long surgical procedures, it is recommended that a small amount of ophthalmic ointment be placed on each eye.

4.2.5 Personal Protective Equipment is mandatory during all surgeries. This includes a mask, bonnet, isolation gown, shoe covers, and sterile surgical gloves.

4.3 Surgery:

4.3.1 Maintain the animal under anesthesia throughout the surgical procedure. Constant monitoring of the anesthetized animal is critical. Periodic observation of vital signs: for example, respiration, color of the mucous membranes (gums or conjunctiva), and a toe pinch reflex is recommended.

4.3.2 Place the animal on a heating pad to help regulate body temperature during all surgeries.

4.3.3 Surgeries must begin with sterile instruments, supplies, and wound closure materials.

4.3.4 A new autoclaved surgery pack should be used for each animal undergoing surgery. All surgical instruments and materials must be handled aseptically. ***(The use of an alternative autoclaved based sterilization should be discussed with the veterinarian before implementation.)***

4.3.5 Sterile drape over the surgical site is recommended to avoid contamination of the incision site. Drapes should cover all exposed body parts. Sterile surgical equipment should be placed on drapes to maintain sterility.

4.3.6 Monitor and evaluate animal's vital signs (see 5.3.1) during surgery every ten minutes.

4.3.7 Close the surgical wound using appropriate techniques and materials. Closure of the skin with non-capillary, non-absorbable material is essential to reduce the risk of post-operative infection.

4.4 Post-Operative:

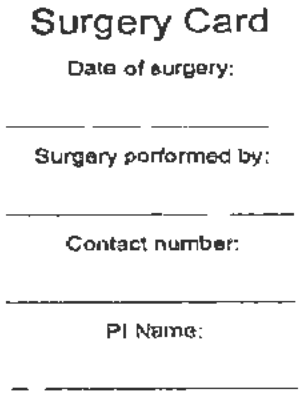
4.4.1 Move animals to a warm dry area after surgery so that vital signs can be monitored during recovery.

4.4.2 Animals must have a heat source during recovery and may require subcutaneous fluids to prevent dehydration.

4.4.3 Observations must be documented.

4.4.4 Return animals to their routine housing but only after the animal has fully recovered from anesthesia.

4.4.5 Place a "surgery" card on all cages which surgery has been performed (as illustrated below). Health checks for these cages will be done first, and then all other cages will be checked.



4.4.6 Observe animals daily for at least three days after the surgery. The incision site should be examined for redness, discharge, or swelling. The animal should also be checked to make sure that drinking, eating, urinating, and defecating are normal.

4.4.7 Reassess for pain and re-administer analgesics if needed (Analgesics must be used post-operatively to reduce pain and stress on the animal).

4.4.8 Remove skin sutures or staples 10-14 days after surgery.

4.4.9 If a post-operative complication such as infection or a lengthy recovery occurs, contact the attending veterinarian immediately.

5.0 References

5.1 The Guide for the Care and Use of Laboratory Animals. (2011). *Surgical Procedures*. Washington D.C.; National Academy Press

6.0 Approvals

This document requires the following approvals.

Name	Title
Carl Olson, PhD	**IACUC Chair**

Name	Title
Sara Andux, PhD, DVM, DACLAM	**Attending Veterinarian**

Name	Title
Heather Bragg	**Institutional Official**

7.0 Document History

Revision Number	Revision Date	Summary of Changes	Author

Morse, Brent (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Monday, December 23, 2019 8:27 AM
To: Heather M. Bragg; OLAW Division of Compliance Oversight (NIH/OD)
Cc: Petervary, Nicolette (NIH/OD) [E]; 'Carl Olson (colson@cnbc.cmu.edu)'; (b) (6) (b) (6)
Subject: RE: OLAW Case A3352-D

Thank you for providing this final report Ms. Bragg. I will forward this information to Dr. Petervary and she will send an official response within a few weeks.

Best regards, Brent Morse

Brent C. Morse, DVM, DACLAM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: Heather M. Bragg [mailto:hbragg@andrew.cmu.edu]
Sent: Sunday, December 22, 2019 9:36 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Petervary, Nicolette (NIH/OD) [E] <nicolette.petervary@nih.gov>; 'Carl Olson (colson@cnbc.cmu.edu)' <colson@cnbc.cmu.edu>; (b) (6)
Subject: OLAW Case A3352-D

Good Evening,

Please find attached a copy of Carnegie Mellon University's response to OLAW Case A3352-D.

Sincerely,
Heather

Heather Bragg
Interim Executive Director
Office of Research Integrity and Compliance
Carnegie Mellon University
5000 Forbes Avenue
Pittsburgh, PA 15213
(b) (6)

DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500, MSC 6910
Bethesda, Maryland 20892-6910
Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

FOR EXPRESS MAIL:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301) 402-7065

November 13, 2019

Re: Animal Welfare Assurance
A3352-01 [OLAW Case D]

Heather Bragg, MSL
Interim Executive Director
Institutional Official
Carnegie-Mellon University
5000 Forbes Avenue, (b) (4)
Pittsburgh, PA 15213

Dear Ms. Bragg,

The Office of Laboratory Animal Welfare (OLAW) has received an animal welfare related concern regarding Carnegie-Mellon University. In order to evaluate these concerns thoroughly and objectively and to determine potential noncompliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy), we are hereby requesting some information. Please direct the Institutional Animal Care and Use Committee (IACUC), avoiding any conflicts of interest, to address the following:

1. Please provide information on how the IACUC addresses animal welfare concerns from known or anonymous sources, and information on any whistleblower policies and protections in place.

2. Please provide information on any IACUC interactions and issues with the laboratories of Dr. Allison Barth and Dr. Aryn Gittis. Specifically, please respond to the following:

    a. The concern that these two laboratories are euthanizing mice with carbon dioxide in overcrowded cages, to the extent that the animals suffocate each other due to high numbers of animals in each cage. The concern states this has happened multiple times and is ongoing. A specific incident on November 6, 2019 was referenced.
    b. The concern that these two laboratories are not confirming euthanasia with a secondary euthanasia method
    c. The concern that euthanized animals are not being disposed of properly (bags of euthanized mice are left in laboratories overnight and allowed to partially decompose). A specific incident in the Barth laboratory on November 2, 2019 was referenced.
    d. Any other recent animal welfare concerns involving these laboratories.

3. Please briefly describe post approval monitoring activities at your institution.

4. Please describe any IACUC approved euthanasia policies at your institution.

Your assistance with this request will help OLAW to assess these concerns as they pertain to the PHS Policy using an efficient, fair and balanced approach. Please provide your responses no later than **December 22, 2019** by emailing olawdco@mail.nih.gov and carbon copying nicolette.petervary@nih.gov. We appreciate your cooperation and look forward to your response. If you have any question, please do not hesitate to reach out to me at 301-496-3133 or via email.

Sincerely,

(b) (6)

Nicolette Petervary, VMD, DACAW
Animal Welfare Policy Specialist
Office of Laboratory Animal Welfare

cc: IACUC Contact