Exhibit 9

9) December 27, 2012 letter from UW IO responding to OLAW's questions.

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UNIVERSITY OF WISCONSIN–MADISON

December 27, 2012

Dr. Axel Wolff, MS, DVM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare, NIH
RKL1, Suite 360
6705 Rockledge Drive, MSC 7982
Bethesda, MD 20892-7982

Dear Dr. Wolff:

The attached documents are in response to your September 19, 2012 letter regarding allegations of non-compliance by PETA (A3368-01, OLAW Case 4P). Upon receipt of your letter, I directed the School of Medicine and Public Health (SMPH) ACUC to address questions and issues raised by OLAW. The SMPH ACUC formed a subcommittee, avoiding conflicts of interest, which reviewed the case and reported back to the full committee. At its December 3, 2012 meeting, the SMPH ACUC approved the subcommittee's report for submission to OLAW.

A summary of the report is as follows:

1. No off-protocol activity occurred.
2. Evidence does not support allegations of inadequate veterinary care and record-keeping. Documentation shows the animal was monitored daily and that there was continuous and extensive action to provide appropriate veterinary care for the animal.
3. Documents support that appropriate ACUC oversight was and is in place, though the investigation does conclude that a protocol amendment to refine the justification of animal numbers is required.
4. Though there is no evidence to support the events alleged to have occurred in 2008, numerous improvements and refinements to ACUC activity and research-animal care have been implemented since that time.

The animal work that was the subject of the specific PETA allegations and your September 19 letter was not funded by NIH. Please let me know if you require further information.

Sincerely,

Name

Name
Title

Attachments: SMPH ACUC Final Summary re: A3368-01, OLAW Case 4P
Laboratory Animal Resources SOP 300: Standard Animal Room Procedures

xc: Name

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UNIVERSITY OF WISCONSIN-MADISON
SCHOOL OF MEDICINE AND PUBLIC HEALTH
LABORATORY ANIMAL RESOURCES

STANDARD OPERATING PROCEDURE

NUMBER: 300 **EFFECTIVE DATE:** September 21, 2012

TITLE: Standard Animal Room Procedures

1. **Environmental Monitoring:**
   - **1.1.** Room air temperature, relative humidity, relative room air pressure, and light cycles shall be maintained in accordance with *The Guide*[1] unless otherwise approved by the IACUC and documented. Room air temperature and relative humidity will be monitored daily via Watchdog by Edstrom or Metasys by Johnson Controls. The environmental data will be stored in a secure database managed by UW DoIt Technical Services.
   - **1.2.** Facility supervisor or designee will be notified when parameters are out of range.
   - **1.3.** See applicable 300 series routine husbandry SOPs for the required room environmental conditions for each species.
2. **Animal Observations:** Every animal room will be checked by LAR staff as described below.
   - **2.1.** **First Check:**
     - **2.1.1.** Animals will be evaluated for health status, food and water consumption, urine and feces production, behavior and general appearance.
     - **2.1.2.** This check is required weekdays, weekends and holidays.
     - **2.1.3.** The first check should be completed in the AM unless a prior exemption from the supervisor is noted.
   - **2.2.** **Second Check/"Consistency Check":**
     - **2.2.1.** Prior to the end of the day, a brief scan of the animal room will be done to ensure that all animals have food and water and are not outwardly in need of immediate attention (i.e. flooded cages, dead animals, obvious room concerns).
     - **2.2.2.** This check is not required on the weekends or holidays.
   - **2.3.** Abnormal or unexpected conditions shall be reported to the veterinary staff in accordance with LAR SOPs 201 or 210.
   - **2.4.** Exceptions to these policies can only be made with prior approval of the veterinary staff.
3. **Feed/Water Animals:** See routine husbandry SOPs for each species as applicable.
4. **Clean/Sanitize Primary Enclosure:** See routine husbandry SOPs for each species as applicable.
5. **Clean/Sanitize Animal Room:**
   - **5.1.** For rooms housing small animals (e.g., mice and rats), floors are swept and mopped at least once a week using a veterinary approved detergent. This should be done additionally as needed.

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[1] *Guide for the Care and Use of Laboratory Animals*, 8th edition.

5.2. For rooms housing large animals (e.g., dogs, pigs, cats, rabbits, NHPs), floors are swept or sprayed down on a daily basis. At least once a week the floors are sprayed down and/or mopped using a veterinary approved detergent. This should be done additionally as needed.

5.3. Sinks are cleaned at least weekly or as needed.

5.4. Paper towel and soap or hand sanitizer supply is checked daily and replenished as needed.

5.5. Pre-exhaust filters are checked weekly and are changed as needed.

5.5.1. When replaced, new filters are labeled with the date of filter change.

5.5.2. Microbial Sciences Vivarium BSL3 and Select Agent suites: pre-filters will be supplied for each suite on a regular basis by the LAR staff but changed and documented by the research staff.

5.6. Barrels (e.g., food, bedding, litter, trash) housed in animal rooms must be changed out and cleaned monthly.

5.7. Totes (e.g., food, water bottle, enrichment device) kept in animal rooms must be changed out and cleaned as they are emptied or monthly, whichever comes first.

5.8. Doors, including doorjambs and knobs, are cleaned inside and outside as needed or at least monthly.

5.9. Walls are cleaned as needed to keep them dust and spatter free.

5.10. Nothing is to be taped to the walls.

5.11. Ceilings, exposed pipes, and light fixtures must be kept clean and dust free.

5.12. Brooms and dustpans must be hung up when not in use.

5.13. Animal rooms shall be kept free of research equipment and supplies except for PPE and a minimal number of exchange cages. Exceptions to this policy may be made by getting prior approval from the facility supervisor in consultation with the veterinary staff.

5.14. Unused equipment shall not be stored in occupied animal rooms containing animals.

6. Animal Room Log Books:

6.1. Every animal room shall have a log book that contains:

6.1.1. A copy of LAR SOP No. 300 and the applicable species-specific 300 Series SOP

6.1.2. Monthly Room Activity Log(s)

6.1.3. Where applicable, a monthly Room Census Sheet for each investigator/requisition.

6.1.4. A Special Husbandry Log with any special instructions; these should be cleared through the area supervisor or lead, contain start and end dates, and be initialed by the Principal Investigator Staff.

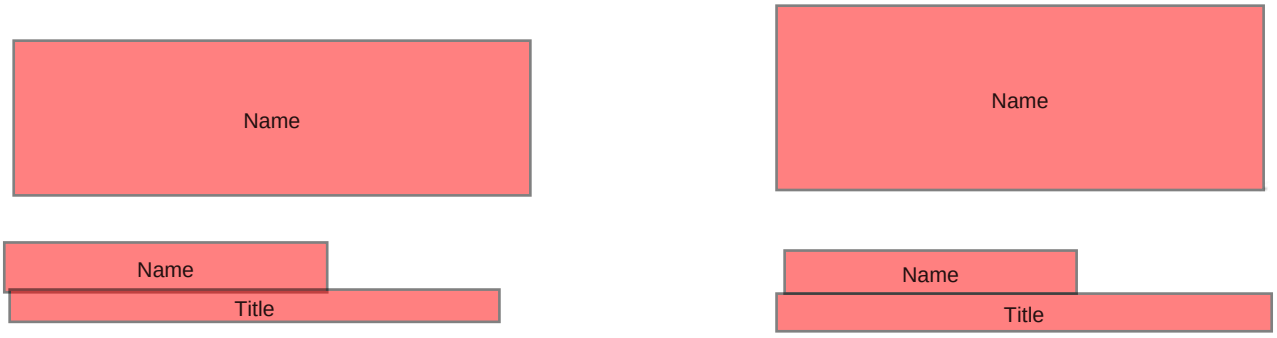
6.2. All animal husbandry activities shall be recorded in the animal room activity log on a daily basis and initialed by the person making the entry.

6.3. The animal research technician assigned to the animal room is responsible for maintaining the animal room logbook.

6.4. Supervisors and/or lead technicians shall check each logbook at least once a month to insure that they are current and properly annotated.

6.5. Where applicable, changes in the number of animals and/or cages are recorded every Thursday or as they occur on the monthly room census sheet for the respective investigator/requisition.

6.6. The monthly room activity log and census sheets shall be turned into the supervisor's office on the first of every month and kept for 3 years.



1. What is the policy on the frequency at which treatment and progress records are updated? Who is responsible for animal observations and making notations in clinical records? Are additional records on an animal's clinical condition kept by the investigator?

- Clinical records by their very nature exist to chart matters of clinical importance. If no veterinary-directed needs exist for clinical entries in the record, no notations are necessarily made. A useful analogy is a person's individual clinical records; the individual's physician does not make entries in the record 365 days per year, but only when there is a medically-warranted need, such as to note a regularly-scheduled physical exam or document and track a significant medical condition. Entries are made in Treatment and Progress records when there is any clinical need to do so; reasons to make entries include documentation of regular physical exams, follow-ups to any observations that might suggest possible clinical concerns, full tracking of any matter of clinical concern, or documentation of administration of any protocol-mandated medication.
- Record types maintained by the School of Medicine and Public Health (SMPH) are:
  i. Treatment and Progress (clinical) records
  ii. Sick Animal Reports and Sick Animal Cards; used primarily to track clinical concerns in rodents
  iii. Daily Observation Logs
    - Treatment and Progress (clinical) records and Sick Animal Reports are separate from daily observation logs; Treatment and Progress records and Sick Animal Reports detail information of clinical relevance, while the daily room-logs show that animal observations are performed by trained animal-care staff each day of the year. Daily observations for cats and all other species at the SMPH are detailed in Laboratory Animal Resources (LAR) SOP 300 (attached).
  iv. Irregular Observations Sheets
    - The SMPH also employs use of an "Irregular Observations Sheet" for USDA-covered species, an innovation implemented by the SMPH veterinary staff since 2008. This system serves as additional layer of animal oversight; briefly, the animals are observed daily by trained Animal Care staff. Any non-emergency irregular (unexpected) observations are recorded on a dedicated irregular-observations sheet posted outside the housing room (note that emergencies are directly reported to veterinary staff as per LAR SOP 210). The veterinary staff checks the irregular observation sheets and follows-up as appropriate. Use of the irregular observation system is detailed in LAR SOP 210; pertinent information from the SOP is as follows:

Irregular Animal Observations Sheets

- **1.1.** Each day, record the date/time of observations and your initials on the "Irregular Animal Observation" sheet.

- **1.1.1.** If there is an irregular observation, record the cage or rack number, animal ID, and observation. Abbreviations located on the bottom of the form can be used.
- **1.1.2.** If the animal is already listed on the sheet, write the observation in the column for the correct day.
- **1.1.3.** If there is nothing wrong in the room, write "OK" on the form for that day.
- **1.1.4.** A new "Irregular Animal Observation" sheet detailing the species and room number will be filled out each week.
- **1.1.5.** Observations and treatments will be recorded in the animal's clinical record as necessary; once the relevant information has been transferred to the clinical record, completed "Irregular Animal Observation" sheets are discarded weekly by the veterinary staff.

- The veterinary staff is responsible for making all clinically-related entries in the Treatment and Progress records, including assessments, diagnoses, and treatment plans. Laboratory staff members can make entries, but these are limited to notes documenting that protocol- or veterinary-directed treatments are performed; for example, if a protocol states that an animal is to receive a dose of analgesic within 12 hours of a surgical procedure, lab staff can administer the analgesic and then are required to write in the clinical record that the analgesic was administered, including documenting the dose provided and the time it was given. The entries must be initialed by the individual performing the activity.
- All laboratories maintain study-specific research records. While these are not intended to be veterinary clinical records, information within these records can be relevant to clinical matters, such as documentation of performance of ACUC-approved surgery on rodents. The Treatment and Progress (clinical) and Sick Animal Reports that are maintained by the veterinary staff are stand-alone documents; that is, these records provide the complete clinical history of an animal, and contain all the information necessary for any veterinarian to understand the medical history, therefore allowing the veterinarian to make informed decisions based on these records.
- In summary, daily observations are performed and recorded by animal care and/or veterinary staff members. If any issues are noted during these observations that are of clinical significance as determined by the veterinary staff, the information is entered in the Treatment and Progress record by veterinary staff members. In this specific case, the daily observation logs and clinical records indicate that the animal in question was observed daily, and when concerns over animal well-being were noted, there was rapid response and timely intervention by the veterinary staff to provide care.

2. There are numerous gaps in the clinical record following initial observations such as facial asymmetry (observed 9/19/08 and the next observation recorded 10/6/08). Indicate what type of follow-up is to be made and by whom when a clinical abnormality is recorded and at what frequency. Is there any additional form of documentation to verify daily observation of animals?

    - As detailed in the response to Question #1 above, notations in the Treatment and Progress records are entered only when there is a clinical need. Any "gaps" in the records correspond to time periods when no issues of clinical concern existed. Also as stated above, animals are always monitored during these times (times when no clinical entries are made in Treatment and Progress records), using Daily Log and Irregular Observation sheets to document the observations. Regarding the specific instance of the "facial asymmetry", the full entry for 9/19/08 states *"Lab staff reported asymmetry to face, left eye/eyelid. PLR (pupillary light reflex) WNL (within normal limits). No ocular discharge, conjunctival swelling or erythema. No facial soft tissue swelling. No blepharospasm. Lab staff thought may have been related to electrical stimulation (i.e. protocol-approved research procedures)"*. Additional notes state the research staff was contacted by veterinarians, the issue was resolved as determined by a veterinarian, and no additional follow-up was required. The animal was clearly examined by a veterinarian, and no abnormality was noted. As such, the case was effectively closed-out. As no further irregular observations were noted for the animal, additional clinical entries were unnecessary until the next observed event that required clinical assessment or intervention (in this case, not until 10/6/08).
    - In general, when a condition is identified that requires continuing clinical oversight, a veterinarian determines the course of action and states this in the Treatment and Progress records. Appropriate follow-up is performed by a veterinarian, or veterinary technician under direction of a veterinarian. The nature of each individual case determines the frequency of entries in the record. Any necessary clinical care is provided by veterinary staff member 7 days/week, 365/year; exceptions to the veterinary staff providing care occur in instances of protocol- or veterinary- approved activity, such as administration of post-operative analgesics, that can be performed by laboratory staff members who are appropriately trained and listed in the protocol.
    - As stated above, Daily Log Sheets and Irregular observation Sheets exist in addition to Treatment and Progress records.

3. Did this specific study outline humane endpoints for removal of an animal from further research activities? Indicate what actions are taken in response to unanticipated adverse events such as chronic infection

    - All animal-use protocols at the University of Wisconsin contain the following questions regarding endpoints that must be addressed by the Principal Investigator:
        - *"Describe how frequently animals will be monitored to ensure they are not experiencing pain or discomfort from your procedures* ***or*** *an unanticipated illness*

or injury not necessary directly related to our research. Describe the criteria or clinical signs (e.g. ruffled fur, hunched posture) that you will use to determine when euthanasia will be performed in these cases"

- *"Describe the **specific criteria** for termination of animals **if experiments could induce chronic disease, tumors or radiation sickness**. These criteria should be described in terms of tumor size, specific animal characteristics or behaviors, weight loss changes, observed clinical signs, etc."*

- In the 2008 animal-use protocol for this specific study, the following statement was present in the protocol to address these questions: *"In order for these experiments to succeed, it is essential that the cats are happy, so much of our efforts are directed towards assuring that they are not in discomfort. If there is an unanticipated illness or injury the lab animal veterinarian is contacted."* Laboratory animal veterinarians have full authority to initiate treatments, remove animals from study, or euthanize animals if needed; therefore removal of animals from further research activity could be determined by the veterinarians if any unanticipated illness or injury was noted by lab staff, animal care staff, or veterinary staff. In the current approved version of the protocol, updated in many areas since 2008, the information is greatly expanded and includes: *"In order for these experiments to succeed, it is essential that the cats are happy so much of our efforts are directed towards assuring that they are not in discomfort. We have been doing recovery chronic surgery for over 15 years and have had few problems with pain or distress with the exception of some low level infection around the wound margins. We clean the wound margins routinely weekly, or more often if needed, with hydrogen peroxide, rinse with sterile water, and apply topical antibiotic ointment if needed as recommended by RARC veterinarians. If the eye coils have detached from the sclera, they will be re-sutured or removed. See Q#17a above for details. In the event of a failure of the head cap, the immediate consideration is to relieve any discomfort for the animal. Typically, this would require that the animal be anesthetized so that any eye or ear coils can be removed and the skin sutured to cover the exposed bone. The skull bone will be carefully evaluated in consultation with the RARC veterinarians to establish whether the head cap can be replaced. X-rays of the skull may be helpful in this regard. During the microelectrode recording experiments, there is always a chance that the electrode may inadvertently puncture an important blood vessel as it is lowered into the brain. Since the penetrations are done through the dura, it is not possible to see the large vessels in order to avoid them. Signs of such damage include subdural hematomas or other bleeding that would be visible through the dura or unusual and new neurologic signs indicating lesions of brain tissue. RARC veterinarians will be consulted in such situations. For the systemic deafening procedure using kanamycin injections, cats will be monitored daily by the lab staff for inappetence, vomiting, lack of grooming, anuria, dehydration, and lethargy and the veterinary staff contacted if any are noted. We will also pay close attention to the possibility that these systemic injections cause undue pain, in which case we will resort to cochlear injections at the time of surgery. If the blood creatinine levels are greater than 2 mg/dL or urea nitrogen is greater than 40 mg/dL with USG lower than 1.025, injections will be stopped*

and a RARC veterinarian consulted. The animal will be euthanized if blood creatinine levels are >8 mg/dL or the BUN is > than 120 mg/dL.

If there is an unanticipated illness or injury the lab animal veterinarian is contacted for treatment."

- Any unanticipated adverse events are reported through a mechanism as described under Q1 above. Animals are then assessed by veterinarians, and an appropriate diagnostic, monitoring and treatment plan is developed based on the nature of the specific case. If determined as being necessary, veterinarians can remove animals from study or intervene to provide humane euthanasia. Although each individual case will vary, plans to deal with chronic infections include culture and sensitivity testing, appropriate antibiotic therapy, and appropriate support activity such as cleansing and flushing of infected areas. In this specific case, a continuous and extensive effort to diagnose, treat and manage the condition took place throughout the fall of 2008. This is clearly detailed in the clinical record, which includes a total of 107 entries in the Treatment and Progress record between 10/22/08 and 12/05/08. Diagnostic and treatment efforts during this time-frame included microbial culture of the site of infection, cleaning and flushing of the site with betadine, topical antibiotic treatment, and systemic antibiotic therapy. As the chronic infection was highly localized, systemic signs of illness were never observed during this time period. Numerous entries of "BAR" (bright, alert, responsive), "Active", "Eating and drinking normally", and "Appears comfortable" exist in the Treatment and Progress record. However, when treatment efforts were deemed to be insufficient to clear the infection, provide for appropriate animal well-being, and allow for continued effective and responsible use of the animal on study, the decision was made to humanely euthanize the animal on December 5, 2008.

4. Comment on the provision of analgesia for this cat while on study and on treatment for the chronic scalp infection. Were the injections of buprenorphine given in response to clinical signs of pain or prophylactically?

- The protocol is written to provide routine post-operative analgesia for the animals. Analgesia that may be necessary outside of the post-operative time-frame is a clinical decision to be determined by the veterinarians. In the 2008 protocol for this specific study, the following post-operative analgesia regimen (in addition to intraoperative administration of an initial analgesic dose) was described: *"These cats may experience some pain (as assessed by criteria that are used in human infants, such as body weight, respiration, mobility and vocalizations) during the recovery period in which additional doses of Ketoprofen or buprenorphine or other analgesic as recommended by an RARC veterinarian is given for up to 2-5 days post-surgery. The veterinarians will be consulted in cases where additional analgesics beyond the initial 2 day period are thought to be needed."* The Treatment and Progress record indicates that post-operative analgesic medication was provided. The re-written and currently approved version of the protocol states: *"Cats are pre-medicated with buprenorphine (0.005-0.01 mg/kg) for sedation and analgesia. This will be repeated at 6 hours for any of the major procedures. Dosing at 6,*

8, or 12 hours IM or bucally will continue for 1-3 days or longer depending on procedure, animal response and recommendation of the RARC veterinary staff. A non-steroidal anti-inflammatory will be given, either ketoprofen (1-2 mg/kg SQ once daily for no more than 3 days) or, meloxicam (1st dose 0.1-0.2 mg/kg SC then oral 0.05 mg/kg PO q24h for 3-5 days) post-surgery or as recommended by an RARC veterinarian. Animals will be monitored at least once daily by the lab staff for a minimum of 3 days post-operatively and any concerns, such as inappetence, hunched posture, failure to groom or respond normally or other indications of pain or distress will be reported to the veterinary staff. The veterinary staff will be informed before the chronic surgeries are performed so that postoperative assessment of analgesia can be monitored." Note that the Principal Investigator uses the terminology of "chronic surgeries" to refer to survival procedures where cats are kept on the protocol for extended times. Though removed from the currently approved protocol, the investigator also has used the terminology of "acute surgeries" in the past to refer to terminal surgeries, where otherwise experimentally naïve cats are euthanized while under general anesthesia.

In this specific case, for humane reasons and in order to follow the principles of the "3 R's" by diligently working to eliminate the need to replace this animal with another, a continuous and extensive effort to diagnose, treat and manage the condition took place throughout the fall of 2008. This is clearly detailed in the clinical record, which includes a total of 107 entries in the Treatment and Progress record between 10/22/08 and 12/05/08. Diagnostic and treatment efforts during this time-frame included microbial culture of the site of infection, cleaning and flushing of the site with betadine, topical antibiotic treatment, and systemic antibiotic treatment using different antibiotics, including Clavamox, Penicillin, and Amoxicillin. As the chronic infection was highly localized, systemic signs of illness were never observed during this time period. Numerous entries of "BAR" (bright, alert, responsive), "Active", "Eating and drinking normally", and "Appears comfortable" exist in the Treatment and Progress record. These veterinary notations from 10/22/08 to 12/05/08 indicate that the cat was comfortable and behaving normally during the time when the localized infection was being treated (also detailed in the response to Q3 above), therefore use of additional analgesic medication was not employed. Nonetheless, when treatment efforts were deemed to be insufficient to clear the infection and provide for appropriate animal well-being, the decision was made to humanely euthanize the animal on December 5, 2008. The clinical record supports the fact that appropriate veterinary care and treatment was utilized to minimize discomfort, distress, and pain, and that when deemed appropriate, the animal was humanely euthanized.

5. The procedure log for 11/21/08 indicates that a two hour procedure was performed under ketamine and acepromazine and that the anesthesia became light requiring additional injection. The approved protocol states that this injectable cocktail would be used only in procedures lasting 30 minutes or less and that all longer procedures would be performed using inhalant anesthetic (isoflurane). Indicate whether the ACUC approved the conduct of a two-hour surgery under injectable anesthesia. If a significant change was implemented without ACUC approval, was any action taken and was this reported to OLAW?

   - The procedure on 11/21/08 was in fact not a surgical or invasive procedure. The animal was anesthetized with ketamine and acepromazine for a non-invasive ABR (Auditory Brainstem Response) procedure; a painless procedure commonly performed on newborn human infants. The record was misinterpreted by the individuals reviewing the records for PETA, and this misinterpretation became one of the centerpieces of PETAs allegations against the University of Wisconsin. The actual note in the clinical record is "Put in chamber"; this refers to placing the sedated animal on a warming blanket on a table inside of an acoustic recording chamber that is located in the investigators laboratory. The acoustic recording chamber is essentially a large sealed box, approximately 6-feet X 6-feet, designed to keep out extraneous noise. PETA mistakenly interpreted a statement referring to placement of the animal inside of the chamber as the surgical placement of a recording chamber onto the cranium of the animal. No surgery took place on this day; no scalp incision was made, no recording chamber was implanted, and no craniotomy was performed. The 2008 version of the protocol, as well as the currently version, do call for deeper planes of anesthesia for surgical procedures, especially for surgical procedures of long duration (greater than 30 minutes). As the procedure in question on 11/21/08 was not a surgical or invasive procedure, a light level of anesthesia or sedation was preferred, and was primarily necessary to keep the animal still during the procedure. As no surgical procedure was performed, and as the sedation/anesthesia used was approved and appropriate for the non-invasive ABR procedure, no off-protocol procedure took place, therefore there was no significant procedural change that occurred without ACUC approval, and therefore there was no violation or issue to report to OLAW.

6. The procedure log for 6/11/08 indicates the anesthetic mask came off and the animal showed signs of waking. If this animal was intubated why did the anesthesia become light when the mask came off and why was the mask used?

   - Regarding procedure on June 11, 2008, the clinical record shows that the animal was intubated at 10:10 am, and placed on isoflurane for the entire procedure. The record also shows that the endotracheal tube was removed at 4:05 pm. The record therefore clearly indicates that the animal was intubated and maintained on appropriate concentrations of isoflurane anesthesia for the entire procedure. One notation made by a laboratory member referring to an anesthetic mask is made at 11:30 am, but these notations do not make sense in the context of the overall record. This event took place over 4 years ago, and it may be difficult to determine the exact origin and meaning of

the notation in the records, but our investigation has revealed that the most likely scenario is as follows: the endotracheal tube became temporarily detached from the tubing of the anesthetic machine during a re-positioning and re-draping of the animal. Momentary detachment of the endotracheal tube from the anesthetic machine is often necessary during surgery when re-positioning of the patient is required. Parameters being monitored such as heart rate and respiratory rate momentarily increased during this time period (to a maximum of 190 and 21 per minute, respectively). The endotracheal tube was reconnected to the anesthetic machine, the concentration of isoflurane was increased for approximately 5-10 minutes, and the animal was returned to a deeper plane of anesthesia before the isoflurane was adjusted down to a normal maintenance concentration. Heart rate and respiratory rate had decreased to 140/min and 4/min, respectively, at 11:45 am. Heart rate averaged 144/min and respiratory rate averaged 8/min for the remainder of the surgery.

- As mentioned in the response to Q1 above, investigators at their discretion can maintain additional notes. The following additional statement was provided by the PI of the study: *"The note in the log about the "anesthetic mask" is also mysterious to us. As far as we can determine, no anesthetic mask was used (it is not part of the standard procedure). We do keep an anesthetic mask handy during these surgeries, but the only condition under which we would use it is if the animal were not intubated or perhaps intubated incorrectly (e.g. if the intubation tube were placed down the esophagus by mistake) and we needed to give the animal isoflurane quickly because it was waking up. There's no indication this happened during this procedure. We have pictures that document the animal had been intubated before the exposure of the bulla and therefore would not need a mask at the time of the note. An additional set of notes that were kept by the lab, parallel to the procedure log, indicate that at the time in question the animal became light because the endotracheal tube was dislodged, presumably because of the need to turn the animal over several times during the exposure of the bulla at this time of the surgery. The animal indeed became light, and the isoflurane rate was increased from 2 to 5% to return the cat back down to a surgical plane. The notes in the procedure log were taken by an undergraduate student who was working in the lab at the time and individual must have thought that the intubation tubes were the same as an 'anesthetic mask'".*
- Our best assessment of this incident is that the animal was being provided isoflurane anesthesia via an endotracheal tube for the entire procedure. Detachment of the endotracheal tube from the anesthetic tubing occurred around 11:30 am, during repositioning and re-draping of the animal. During this time period, anesthesia momentarily lightened. The endotracheal tube was quickly re-connected to the anesthesia-machine tubing, and isoflurane concentration was increased to return the animal to a deeper plane of anesthesia. Once this plane was achieved, the procedure resumed. The notation about a mask coming off was made by an observer and note-taker, not an individual overseeing the actual anesthesia, and this individual made an imprecise entry in the record, misinterpreting the endotracheal tube/anesthesia machine tubing connection as an "anesthetic mask" apparatus. It is not uncommon for endotracheal tubes to be briefly disconnected during surgical procedures; in this case

the situation was dealt with appropriately, there was never an indication of harm to animal well-being, and the procedure and the recovery were uneventful. As this aberrant entry has resulted in many questions about the anesthetic record from this day, the laboratory staff and the veterinary staff will use this example as a training opportunity to educate researchers and veterinary staff members to refine and improve surgical and anesthetic record-keeping practices.

7. Provide an explanation of the adequacy and competency of staff involved in the surgeries and their ability to maintain an appropriate plane of anesthesia during the procedures.

- The following training is required of all individuals who handle research animals in any way at the University of Wisconsin:
    i. An Animal User Orientation Course; an on-line module that provides an overview of the rules and regulations regarding the use of animals in research
    ii. A Medical Records course (newly developed since 2008)
    iii. Occupational Health and Safety courses
    iv. Species-specific training for all species that an individual may be identified as working with on the animal-use protocol; one example is a course entitled "Biomethodology of the Cat"
- The following is additional training required of any individual who may perform surgical procedures.
    i. Laboratory Animal Surgery course; a hands-on course that teaches concepts of anesthesia, sterile technique, instrument handling, tissue handling, and suturing.
- An electronic database of training is maintained by the Research Animal Resources Center (RARC) to assure that individual listed in a protocol have completed the proper training; the veterinary staff and/or ACUC are alerted to individuals who have not completed the required training.
- The PI has more than 40 years surgical experience with procedures using cats and non-human primates as described in the approved protocol. All individuals listed in the protocol have completed all the required RARC training, and have experience with surgery or must be directly trained and supervised by qualified laboratory staff.
- In this specific case, RARC veterinary staff assisted with anesthesia induction until a surgical plane of anesthesia was achieved. Since 2010, the veterinary staff has increased anesthetic and procedural oversight; a qualified representative of the University's veterinary care operation is present for the entire procedure, providing and overseeing anesthesia until the animal and extubated and recovering.
- The RARC veterinarians routinely consult with available specialists. Anesthesia consults have been on-going with board-certified veterinary anesthesiologists from the UW School of Veterinary Medicine since 2010, including in regard to this specific protocol. Recommendations from anesthesiologists are implemented or used to refine anesthesia practices for procedures detailed in animal-use protocols.
- For all surgeries, the aim of maintaining an appropriate plane of anesthesia is to keep the animal anesthetized sufficiently enough to ensure there is no pain perception but

not so deep as to present a danger of not safely recovering from anesthesia. The surgery and anesthesia records indicate an appropriate plane of anesthesia was and is utilized for the surgeries performed under this protocol, and that anesthetic recoveries have been without adverse event.

8. Provide information on the type of justification investigators are to provide the ACUC for the species and numbers of animals requested. What type of statistical justification is required?
   - All animal-use protocols contain the following questions that must be addressed by Principal Investigators:
     - *Specifically justify why you chose the species for your work, such as the appropriateness of the species for your proposed work. Cost considerations are not justifications.*
     - *Explain how the number of animals required was determined and justify that need. Include all control animals and breeding colony animals in this discussion. A table may help clarify different experimental groups or studies and the specific numbers needed for each. Include any statistical analysis used (e.g. power calculations) in determining the animal numbers.*
   - The ACUC examines the justification for species used on a case-by-case basis. The ACUC also examines and approves numbers justifications on an individual basis, as the specific type of explanation and analysis that makes sense for each separate instance varies from study-to-study.
   - In instances where the ACUC determines that questions exist or a more complete explanation is needed concerning species chosen or numbers justification, the PI is directed by the Committee to provide additional justification, detail, or statistical information in a required re-write and re-submission of the protocol.
   - In this specific case, the following justification is provided by the PI in the protocol regarding species chosen: *We use cats for the following reasons: the physiological, anatomical and psychophysical characteristics of their auditory system are very similar to those of humans and higher primates (which makes it likely that our results, except for those related to movable pinnae, are also applicable to humans), their auditory system has been extensively studied by others such that most of our understanding of auditory physiology derives from studies in the cat, the relevant parts of their brain are relatively easily accessible, and they are not endangered or in short supply. The other animal species that have been extensively used in studies of sound localization are guinea pigs, gerbils, chinchillas, and barn owls. The rodents are not good models for studies of localization because the behavioral evidence indicates that their localization acuity is considerably less than that of predators like the cat, barn owl or human. For prey, they need only determine the general direction of a sound source, not its precise location. Barn owls are not good models since they are so highly specialized (they are the only animals known to phase lock to frequencies above 3-4 kHz and they do not move their eyes) that results may not apply generally to other animals.*

- In this specific case, the following justification is provided by the PI regarding animal numbers: *We have been studying the auditory system for over 30 years here at UW-Madison and the number of animals requested represents an average taken over a number of years. We make an extensive effort to gather as much data from each animal as possible: the chronic cats are kept for many months, even years. Ultimately, the number of cats needed is determined by the scientific aims of the experiment and is governed by many different considerations: a large number of neurons need to be sampled in order to gain statistical viability and to meet the demands of critical reviewers for our manuscripts, there are practical limits to the number of neurons we can study in each cat as it takes an hour or more to characterize each neuron, and every experiment does not work for many different reasons. It is not possible to state how many neurons are required to reach statistical viability since that depends upon the questions that we are addressing and the differences we see between different neurons in any given experiment. For example, if all the neurons consistently show a strong effect, then relatively few neurons are needed to reach significance and therefore few animals are needed; but if there is considerable variability between the neurons then many more cells are required to demonstrate the presence or absence of an effect, i.e. in a t-test. Thus, it is not only more practical but also more realistic to justify the number of animals based upon past experience. While we have averaged about 2-3 chronic cats/year, this number is quite variable, depending upon the experiments that are being done at any given moment. This number allows us to collect enough data to keep up a productive publication record that ensures our constant funding from NIH over these 30 years. We endeavor to use as few animals as possible; and the chronic experiments allow us to use the same animal many times.*

  During a recent USDA APHIS investigation of this specific case, it was determined that the sentence "*This number allows us to collect enough data to keep up a productive publication record that ensures our constant funding from NIH over these 30 years*" -- while never intended to be a justification for animal numbers needed -- can be interpreted as being inappropriate, as matters relating to publishing and funding can never be considered to be a scientific justification for the utilization of animals in research activity. The ACUC has directed the PI to submit a protocol amendment with a re-write of the numbers-justification section, which must include removal of this statement. In order to continually improve protocol-review activity, specific Committee discussion about this action will be led by the ACUC Chair and Senior Program Veterinarian.
- The Principal Investigator has also provided this additional statement regarding animal numbers:

  - *In neurophysiological or behavioral experiments, which are the focus of our research, it is not possible to predict the number of animals needed based on statistical considerations of the numbers of animals. In electrophysiological experiments, it is the number of neurons that is important and this number can be highly variable: one animal may yield 10 neurons while another one may yield 110. This degree of variability is not uncommon. Moreover, the number of*

neurons needed also depends on the questions being asked and the responses of the neurons studied. If responses to a particular variable are being studied and all of the neurons show a strong tendency to respond in a certain way to that variable, then not many neurons need to be recorded for statistical reliability. On the other hand if the responses are highly variable, then many more neurons are needed to attain (or not) statistical significance.

- Due to the specific nature of this study, where the number of neurons and not the number of animals is the determining factor, the justification provided by the Principal Investigator was found to be acceptable by the ACUC. The numbers justification narrative will be reexamined by the ACUC in the required amendment to be submitted by the PI, and the narrative will be refined if deemed necessary by the Committee.

9. Are these studies ongoing? If so, has the ACUC determined that all staff is appropriately trained, that anesthesia is appropriately applied, that animal observations are adequately documented, that humane endpoints are in place, and that animal numbers are appropriately justified? Provide any information on the application of refinement, reduction, or replacement to these types of studies.

- This protocol remains active. The cochlear implant experiments have not been done since 2009. The other behavioral sound localization experiments are on-going. As mentioned in responses to several of the preceding questions, the animal protocol has been rewritten: an extensive re-write of the protocol was specifically requested by the SMPH ACUC in August of 2010, and the most recently amended version was approved by the ACUC in August of 2012. All personnel listed in the protocol have completed all required training.
- The anesthesia regimen has been updated in the re-written protocol. A board-certified anesthesiologist from the UW School of Veterinary Medicine was consulted in the refining of anesthetic practices for the procedures detailed in the animal-use protocol. Consultations took place in 2011 and 2012. One specific example of an update to the anesthesia regimen is that the combination of ketamine and dexmedetomidine has been added as an option for anesthetic induction.
- A system of Irregular Observation sheets has been implemented (described in the response to Q1). These sheets are present at every room that houses USDA-covered species, and are available for inspection by ACUC members during semi-annual facilities inspections. Room logs documenting daily checks of animals in all housing locations are also readily available for inspection by ACUC or veterinary staff members.
- As detailed in the response to Q3, the protocol contains new entries to describe study end-points, which have been approved by the ACUC.
- As stated in the response to Q8 above, appropriate justifications for the species used and animal numbers are required in animal-use protocols. The justifications provided by the Principal Investigator in the re-written protocol were found to be acceptable by the Committee. Nevertheless, as previously indicated, an amended statement of numbers-

justification from the PI will be requested by the Committee, including removal of the sentence referring to funding and publications.

- The laboratory continues to refine and improve their procedures, as the research project and the protocol have undergone changes and refinements since 2008. As an example, an acute experimental procedure that involved naïve animals in a terminal procedure under anesthesia has been removed, decreasing the number of cats needed; this is evident in the current version of protocol, where the total number of animals to be used over a 3-year period is set at a maximum of 15. Note that the actual number of animals the laboratory has used over the last 5 years is considerably less than that estimated in the protocols: since 2008 the laboratory has used 8 cats in 4 years, an average of 2 cats/year. Another example is an expanded explanation of the post-operative analgesia regimen as is detailed in the response to Q4 above.
- The veterinary staff has implemented multiple refinements of its operations since 2008 that have been applied across the entire SMPH animal program, ranging from increasing the number of veterinarians and veterinary technicians at the SMPH to requiring veterinary pre-review of all new protocols to requiring increased anesthesia consultation and oversight. In this specific case, as previously noted, a board-certified anesthesiologist from the UW School of Veterinary Medicine was consulted in the refining of anesthetic practices for the procedures detailed in the animal-use protocol. The veterinary staff also consults with other experts on campus, such as board-certified ophthalmologists, veterinarians from the Wisconsin National Primate Research Center, and microbiologists.
- Another example of refinement and improvement in veterinary operations is that in 2010 and 2011, the SMPH and RARC established and equipped new additional dedicated treatment and critical-care rooms for USDA-covered species within animal holding areas, including in the hallway housing the animals associated with the specific protocol that is the subject of the current inquiry.
- The ACUC has implemented multiple refinements to its protocol review and oversight activity. As with refinements to veterinary operations, these improvements represent an overarching change that applies to the entire research animal program and not only to the specific case in question. An SMPH ACUC subcommittee was formed in 2010 to develop a recommendation for changing the protocol form to better address the questions of unnecessary duplication and the search for alternatives to potentially painful or distressful procedures. The new protocol questions were adopted by the full Committee, and all SMPH protocols were examined and by the Committee and updated by the principal investigators as needed. Also, in 2011, the SMPH ACUC Chair and Senior Program Veterinarian scheduled meetings with School of Medicine academic departments as a way to directly interface with investigators and discuss the changing regulatory environment, as well as the role and services of the ACUC and the veterinary staff. Educational sessions for ACUC members during scheduled meetings have also been implemented; a series of training sessions took place in 2011 to inform Committee members about significant changes in the 8th edition of *The Guide for the Care and Use of Laboratory Animals.*

- An improved interface also now exists between the RARC training staff and the ACUC to help assure that the training program for personnel working with animals is adequate. For example, a representative from the training staff regularly attends semi-annual ACUC program reviews. Two additional trainers have also been added to the RARC training staff in the past year.
- In keeping with the statement on p. 121 in the 8th edition of the *Guide* "It is therefore essential that personnel caring for and using animals be trained in species-specific and individual clinical, behavioral, physiologic, and biochemical indicators of well-being", the SMPH veterinary staff and the SMPH Laboratory Animal Resources training staff collaborated on developing training seminars for animal-care personnel on recognizing signs of pain or distress in both USDA- and non-USDA-covered species. The training sessions are part of an overall commitment by the SMPH animal-care program to assure animal well-being in all areas of the program, not just for studies similar to the sound-localization study. A poster presentation on the development and implementation of this training program was presented at the 2012 AALAS National Conference.

10. Provide any additional relevant information to this specific case or any other similar study using cats in hearing studies.
    - All information relevant to this case is detailed in the answers to questions 1-9 above. There are no other protocols on campus involving cats and hearing studies.

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Friday, December 28, 2012 7:34 AM
To: ndary Personn@grad.wisc.edu'
Subject: RE: Letter/Attachments from Name UW-Madison

Thank you for this letter, 2ndary Personnel I will review carefully and respond to the IO soon.
Axel Wolff, M.S., D.V.M.
Director, Division of Compliance Oversight OLAW

-----Original Message-----
From: 2ndary Personnel @grad.wisc.edu [mailto:2ndary Personnegrad.wisc.edu]
Sent: Thursday, December 27, 2012 1:20 PM
To: Wolff, Axel (NIH/OD) [E]
Cc: Name Name Name Name Name
Subject: Letter/Attachments from Dan Ulrich, UW-Madison

Dear Dr. Wolff:

Please see the attached letter and documents from Name UW-Madison. Thanks.

Name

--

2ndary Personnel
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Graduate School
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Madison, WI 53706
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