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

October 15, 2018

Re: Animal Welfare Assurance
A4051-01 [OLAW Case V]

Ms. V. Kay Holt
Deputy Director, National Health and
Environment Effects Research Laboratory
U.S. Environmental Protection Agency
EPA-MD-B305-01, 109 T.W. Alexander Drive
Research Triangle Park, NC 27709

Dear Ms. Holt,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your October 11, 2018 letter reporting an instance of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at the National Health and Environmental Effects Research Laboratory, US EPA, following up on an initial telephone report on July 12, 2018. According to the information provided, OLAW understands that a chemical exposure study in pregnant rats resulted in unexpected neonatal mortality and that the days the dams received test chemical gavage was different from what was described in the protocol. Additional deviations included not amending the protocol to change the pain category and to state that death was an endpoint, not informing the Attending Veterinarian (AV) or Institutional Animal Care and Use Committee (IACUC) about hind limb discoloration in some pups, and not stating in the protocol that some pups were cross-fostered.

The corrective actions consisted of stopping data collection from one cohort of rats and euthanizing them, amending the protocol to the correct pain category, withholding journal submission of data acquired using unapproved procedures, counseling the Principal Investigator (PI) on what constitutes a significant change to a protocol, distributing a copy of the protocol to all laboratory staff involved, having the PI keep the IACUC Chair and AV informed about the study and any problems and submitting amendments prior to initiating significant changes, having the laboratory post an animal health check list, placing the laboratory under enhanced monitoring, and retraining the laboratory staff.

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. If this study was NIH supported, please also inform the funding component and ensure that the grant is not charged for any unauthorized animal activities. Thank you for keeping OLAW apprised on this matter.

Sincerely,

[redacted]

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

cc: IACUC Chair

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory Research Triangle Park, NC 27711

OFFICE OF RESEARCH AND DEVELOPMENT

October 11, 2018

Dr. Brent Morse, Director
Division of Compliance Oversight
Office of Laboratory Animal
Welfare Rockledge One, Suite 360
6705 Rockledge Drive, MSC-7982
Bethesda, MD 20892-7982

Dear Dr. Morse:

The purpose of this letter is to describe a noncompliance issue at our institution (OLAW Assurance number: A4051-01), which was identified July 2018, and to inform your office of the IACUC's disposition of the issue. Your office was given a preliminary notification of the incident via telephone contact to Dr. Axel Wolff from our IACUC Chair (Dr. Michael G. Narotsky) on July 12, 2018.

Briefly, the noncompliance involves a two-block rat study with test chemical exposure to pregnant dams. Although neonatal mortality was unexpectedly observed in Block 1, the principal investigator (PI) did anticipate neonatal mortality in Block 2 and proceeded to conduct Block 2 without formal IACUC notification of the Block-1 mortality or re-classification of the pups' pain category. When researching the details of this case, it was discovered that two protocol modifications were enacted without prior IACUC approval – the procedures were discussed openly and appear to be examples of protocol drift.

A more detailed explanation of the events involved, and the corrective actions taken by the PI and the IACUC are given below. In September 2017, the IACUC approved a protocol amendment to add a test chemical to the protocol and to conduct a two-block study with this test chemical. In March 2018, Block 1 of the study was initiated by the PI's research staff. The dams received test chemical by gavage on gestation day (GD) 8 to postnatal day (PND) 3, rather than on GD 8 to 18 as stated in the approved amendment. During this dose-range-finding phase, it was found that litters at ≥62.5 mg/kg experienced neonatal losses, generally within 1 day after birth. Deaths were reported to the Attending Veterinarian (AV) and the IACUC Chair, although the dose-dependent nature of the mortality was not communicated to the AV. Complicating interpretation of the data, an older control litter was missing and speculated to be the result of an adverse housing event, but pup mortality and cannibalization by the dam could not be ruled out.

While Block 1 was still in progress, the IACUC Chair and PI discussed the need for the PI to submit an amendment to reclassify the mortality cases from Pain Category C to Pain Category E. In June 2018, such an amendment still had not been submitted, yet the lab initiated Block 2 of the study. The dams were treated using the same dosing schedule and a subset of the dose levels (0 and 125 mg/kg) used in Block 1. Based on Block 1 results, pup mortality was expected at 125 mg/kg; this endpoint was not approved in the IACUC protocol or amendment. Further, to test whether the effect was maternally/lactationally mediated, newborn pups were cross fostered (i.e., pups from treated dams were moved to control dams and vice versa). This cross-fostering procedure was also not included in the approved protocol or amendment. As expected, pups in the treated groups had high neonatal mortality. Two of the four treated litters each had two pups with an unusual finding, blue discoloration of the right hindleg. Two pups from one litter were euthanized and preserved for histopathology; the pups from the other litter had died before the research staff could euthanize. The finding of right hindlimb discoloration was not reported to the IACUC or AV. The lab had observed an isolated case of right hindlimb discoloration in a dead pup in Block 1, and although considered an oddity at the time, the finding prompted the lab to be alert for this characteristic in Block 2.

On July 11, 2018, the IACUC Chair discussed these events with the PI. On July 12, 2018, the Chair submitted a preliminary report of noncompliance by telephone. On July 13, 2018, the AV and Animal Resources and Research Support (ARRS) Director discussed these issues further with the PI. Following this discussion, data collection from the Block 2 animals was promptly halted at the PI's direction and all animals in this cohort were euthanized. The PI has since submitted an amendment reclassifying 72 pups (from 14 litters) in Block 1 from Category C to Category E; this amendment was approved by the IACUC on August 15, 2018.

Initially, the noncompliance investigation focused on the transgressions of Block 2, i.e., the expectation of neonatal mortality and the cross-fostering procedure, both without prior IACUC approval. However, when the investigation revealed that Block 1 was also noncompliant (using an unapproved dosing regimen), the PI withheld journal submission of the internally cleared-to-publish manuscript reporting their findings with the test chemical, including the unexpected Block-1 neonatal mortality.

The IACUC has requested that the PI prepare a written explanation of how this series of events occurred and measures to prevent recurrences. Toward that end, the IACUC requested the PI prepare a command (what is the PI's role?), control (Who is responsible for day to day operations?), and communication (How does the information flow within the project?) document for his animal protocols to assure that the PI and research staff know the contents of the protocols and amendments.

The PI has responded to the IACUC's request. The response included the following points:

- The intended dosing period for the dams was indeed GD 8 to PND 3; however, when preparing the amendment, the PI inadvertently copied text from an earlier study so that the amendment incorrectly stated that exposure would be from GD 8 to 18. The study was initiated several months later and the error was not caught until after Block 2 was in progress.

- The PI felt it was important to demonstrate that the unexpected neonatal mortality observed in Block 1 be reproducible before publishing the findings on this chemical of public concern. Based on industry studies with the chemical and the lab's studies when dosing on GD 14-18, there was no expectation of neonatal mortality in Block 1. The PI's main reason for conducting Block 2 was the concern that the neonatal mortality may have been unrelated to treatment with the test chemical. This concern was based on 1) the disappearance of a healthy Block-1 control litter, and 2) historical episodes of unexplained pup mortality (including controls) that have occurred over the years.
- The PI was unaware that an extension in the dosing period required an amendment.
- Similarly, the PI was unaware that the cross-fostering procedure required an amendment, arguing that this is a common procedure that does not have any adverse effects on pup health or welfare at this age window.
- The PI is now aware that both the cross-fostering procedure and extending the dosing period are considered significant modifications that require an amendment and IACUC approval.
- The PI and research staff are taking the following steps:
    - Lab staff will distribute and review protocols and amendments to all individuals in research team involved in animal work. PDFs will be distributed. Hard copies will be kept in a central data book.
    - The PI will continue to inform the IACUC Chair and AV of all results of the study and study plans.
    - The PI will submit amendments to allow extended dosing periods prior to initiating the studies, so that similar situations will not recur.

In addition, the IACUC is taking these further corrective actions:

- The PI will be required to contact the following personnel 1 week before initiating a study: AV, IACUC Chair, IACUC Administrator, ARRS Director, and IACUC branch representative.
- The PI will be required to immediately inform the following personnel of any abnormal or unusual effects: AV, IACUC Chair, IACUC Administrator, ARRS Director, and IACUC branch representative.
- The research team will be required to post a check list for daily animal checks; this check list must be plainly visible in the animal suite to the veterinary and animal care staff as well as the PI and research staff.
    - If unusual issues arise, then animal checks will increase to 2× to 3× per day (including weekends) or as otherwise advised by the AV.
- The IACUC will implement increased monitoring over a 6-month period of animals, check lists, and animal procedures, to include unscheduled visits by IACUC members, the AV, IACUC Administrator, as well as scheduled post-approval monitoring.
- Refresher training is required of all staff on protocol for regulatory and welfare issues.
    - In-house training will include topics: significant vs. minor changes to protocols (i.e., when is an amendment is required), variations in the amendment process (e.g., direct-to-DMR, full committee review, VVC), and recognizing normal vs. abnormal animal appearance (i.e., when to contact the veterinarian). This training will be required for this lab and will be a requirement for all animal users on campus.
    - Pertinent AALAS Learning Library online modules will also be required as refresher training for this lab.

The IACUC has tabled consideration of any amendments for new research proposed by this PI until we hear feedback from OLAW regarding this report.

We hope these measures satisfactorily address the issue. Please contact Michael Narotsky [redacted] or narotsky.michael@epa.gov) if you have any questions or comments.

Sincerely,

[redacted]

V. Kay Holt
Deputy Director,
NHEERL Institutional
Official

[redacted]

Michael G. Narotsky, PhD
NHEERL IACUC Chair

cc:
Dr. Leslie Jarrell, Attending Veterinarian
Jaimie Graff, Director of Animal Resources and Research Support
AAALAC, International

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Monday, October 15, 2018 9:51 AM
To: Narotsky, Michael
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Final report of noncompliance incident

Thank you for this report, Dr. Narotsky. We will send a response soon.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

From: Narotsky, Michael [mailto:Narotsky.Michael@epa.gov]
Sent: Thursday, October 11, 2018 12:57 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Graff, Jaimie <Graff.Jaimie@epa.gov>; Jarrell, Leslie <Jarrell.Leslie@epa.gov>; Holt, Kay <Holt.Kay@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>
Subject: Final report of noncompliance incident

Dear Dr. Morse,

We are submitting the attached PDF as the final report pertaining to a recent incident of noncompliance at our institution (OLAW Assurance number: A4051-01).

Please let me know if you have any questions or comments.

Sincerely,
Mike Narotsky

Michael G. Narotsky, Ph.D.
NHEERL IACUC Chair
Research Toxicologist
U.S. Environmental Protection Agency
Endocrine Toxicology Branch, Toxicity Assessment Division
NHEERL (MD B105-04)
Research Triangle Park, NC 27711
[redacted](fax)
narotsky.michael@epa.gov

A 4051 - V

Office of Laboratory Animal Welfare

OLAW

Initial Report of Noncompliance

By: aw

Date: 7/12/18 Time: 3:30

Name of Person reporting: Michael Neurotsky

Telephone #: [redacted]

Fax #: [redacted]

Email:

Name of Institution: US EPA

Assurance number: A4051

Did incident involve PHS funded activity? _____

Funding component: _____

Was funding component contacted (if necessary): _____

What happened?

Toxicity study w/ pregnant rats, some pups died. Work done not on protocol, death as endpoint.

Species involved: Rats

Personnel involved:

Dates and times:

Animal deaths: Yes

Projected plan and schedule for correction/prevention (if known): _____

Amend protocol

Projected submission to OLAW of final report from Institutional Official: August

OFFICE USE ONLY

Case #_____