DEPARTMENT OF HEALTH & HUMAN SERVICES

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FOR US POSTAL SERVICE DELIVERY:
Office of Laboratory Animal Welfare
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Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

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Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
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April 7, 2020

Re: Animal Welfare Assurance
A3231-01 [OLAW Case 1Y]

Dr. Christopher Agnew
Associate Vice President for Research
Purdue University
703 Third Street
West Lafayette, IN 47907-2040

Dear Dr. Agnew,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your April 6, 2020 letter reporting two incidents of noncompliance with the PHS Policy on the Humane Care and Use of Laboratory Animals at Purdue University, one which resulted in the deaths of dogs. According to the information provided, OLAW understands that on 2/12/20 a study dog was anesthetized and placed on an electric heating board (sow board) covered by blankets. The dog suffered apparent thermal injuries and was treated by study personnel and veterinary staff and recovered from the injuries.

Corrective and preventive actions included the PACUC directing the lab to discontinue using the sow board and only use protocol-approved warming devices.

The second incident involved the injection of a study drug that was prepared by an outside company into three dogs. Due to short supply of the drug, the lab also used drug that was synthesized within the lab. No adverse effects were expected. A second dose was administered later that day. Two dogs received the planned dose but the third dog only received a partial dose due to movement of the dog. The next day two dogs were depressed and one vomited. Blood work indicated kidney failure. The three dogs were admitted to the ICU. On February 28th it was determined that unknown impurities in the study drug mixed within the lab were probably responsible for the kidney failure. The dogs were euthanized on March 3rd and necropsy revealed both renal and myocardial injury.

Corrective and preventive actions included the lab using a reference standard to evaluate all drug samples going forward. The PACUC also requested that myocardial and renal damage be monitored in dogs receiving the drug and that the protocol be clarified as to the source of the drug.

OLAW believes that the corrective and preventive measures put in place by Purdue University are consistent with the provisions of the PHS Policy on Humane Care and Use of Laboratory Animals. It was noted that neither activity was supported with PHS funds. Although this activity was not PHS funded, the application of the expectations of the PHS Policy across the animal care and use program reduces any potential appearance of a double standard.

We appreciate being informed of these incidents and find no cause for further action by this office.

Sincerely,

Brent C. Morse -S Digitally signed by Brent C. Morse -S
Date: 2020.04.10 09:17:07 -04'00'

Brent C. Morse, DVM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC Contact
Dr. Robert M. Gibbens, USDA, APHIS, AC

A3231-14

PURDUE
UNIVERSITY

OFFICE OF THE EXECUTIVE VICE PRESIDENT FOR
RESEARCH AND PARTNERSHIPS

ASSOCIATE VICE PRESIDENT FOR RESEARCH,
REGULATORY AFFAIRS

March 26, 2020

Assurance #D16-00147

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

Dear Dr. Wolf:

I am providing information to the NIH/Office of Laboratory Animal Welfare regarding two separate incidents that led to the deaths of dogs on research protocols. While neither of these studies are supported by NIH funds, we feel it necessary to report the incidents. They have also been reported to the USDA.

Adverse event on protocol 1812001828: client-owned dog:
This study is investigating the role of omeprazole vs placebo in the development of GI signs in dogs undergoing surgery for intervertebral disc herniation. An adverse event (death) was reported by the PI in a recently enrolled study dog (client-owned). This is not thought to be related to the study drug and the investigators remain blinded (for this and all other enrolled dogs). The case details are briefly outlined below.

Vala Langston (MR#810-329), 9yo F Dachshund, was presented to Emergency Care Clinic on 2/27/20 for acute onset paraplegia with intact pain perception. She was enrolled in the study and subsequently underwent MRI and hemilaminectomy surgery that night with no complications noted. She was slightly worse neurologically post-operatively so a CT scan was performed under sedation on 2/29/20 which showed good compression at the surgical site. Vitals were within normal limits from intake through late morning of 3/1/20 at which time she acutely decompensated and suffered cardiorespiratory arrest. CPCR was instituted and she was revived but the owners elected humane euthanasia and consented to necropsy. Preliminary gross findings showed cardiomegaly with valvular endocardiosis, pulmonary congestion/edema and focal softening of the spinal cord (at surgical site). Histopathology is pending. Current suspicion regarding cause of decompensation/death is acute, congestive heart failure secondary to previously subclinical heart disease or aspiration pneumonia with less likely considerations being delayed anesthetic reaction or other as yet undetermined pathology. No GI signs were noted throughout hospitalization and the acute demise is not thought to be related to the study drug.

The Purdue Animal Care and Use Committee (PACUC) met on March 18, 2020, to discuss this adverse event and felt that no further actions were needed regarding this unfortunate incident.

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Adverse events on protocol 1802001694: research dogs:
As described by the Principal Investigator, Dr. Low:

Adverse event #1: On 2/12/2020, a dog ((b) (6)) was anesthetized as planned, per protocol 1802001694, for blood pressure monitoring while receiving escalating drug doses under anesthesia. To maintain body temperature while under anesthesia, he was placed on 2-3 blankets which were covering an electric heating board ("sow board"). This dog had previously been implanted with a telemetry device that transmitted blood pressure, ECG, and temperature to a nearby computer in real time. It was assumed that the indwelling temperature reading from the telemetry device would be more accurate than an oral or rectal temp, so temperatures reported via the telemetry device were primarily monitored throughout the 2 hours of anesthesia. However, for comparison, a rectal temperature was taken before premedication and again at the start of anesthesia. Rectal temperature before pre-med was 100.8 and at the start of anesthesia was 97.8. Approximately 15 minutes later the temperature reported by the telemetry device was 97.8, an exact correspondence to the previous rectal temp. We had no problems in telemetry temperature accuracy or overheating with the blanket covered heating boards on the previous 2 dogs anesthetized the day before. With that in mind, as well as the initial matching rectal and telemetry temperatures, all subsequent temperatures were taken via telemetry. During the last 15 minutes of anesthesia and continuing into recovery, (b) (6) respiratory rate continued to increase. This was initially suspected to be a response to light anesthesia or a possible drug reaction, but upon reflection may also have been a response to increasing body temperature. A rectal temperature was taken during recovery, after extubation. Rectal temp was 102.5 (high end of normal). The most recent telemetry temperature was 96.7, taken within the previous 15 minutes. It was noted that his abdomen and testicles appeared red which was when study personnel became concerned of overheating and possible burn from the heating board. In response, staff immediately wrapped bags of ice in towels and placed them along the aggravated skin until rectal temperature decreased and panting returned to a more normal respiratory rate. Approximately 15 minutes later: T101.0 P-140 R-24. The bags of ice were removed and triple antibiotic ointment and aloe vera gel were applied to the affected area. Per LAP veterinary staff, he was also administered 25 mg carprofen PO. Follow up checks the next couple of days showed no visible skin damage to abdomen or testicles and it was stated that there was no cause for additional concern. On 2/24/2020, it was noted that (b) (6) had a wound on the front of his left hindlimb. The PCL staff and LAP veterinary staff sedated him and cleaned, examined, sutured, and bandaged the wound. A small section on the medial aspect of the wound appeared to be necrotic tissue, which was removed by (b) (6) before suturing. The skin of the left inguinal area/flank also had a couple inches of dark, possibly necrotic skin. TAO was placed on both injuries, which based on their location, is assumed are latent effects of a burn caused by prolonged, indirect contact with the heating board. Per LAP veterinary staff, after suturing he was to receive 25 mg carprofen BID for 2-4 days and antibiotic SID (250 mg cephalexin PO and/or 200 mg cefazolin SQ after sedation for re-suturing) for 7 days. Due to (b) (6) frequently slipping his bandage and tearing his sutures, this dosing regimen has been restarted whenever his wound has had to be sutured closed again. During bandaging, it has also been noted that a small "hotspot" has developed on the dorsal aspect of the bandaged foot due to the several times he has chewed off the bandage and exposed that part of his leg. The spot is dried off at every bandage change and TAO applied before rewrapping it.

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Adverse event #2: Study personnel wanted to compare a targeted version of the drug to the original formulation of the drug reported in the literature in order to better predict the effective dose of the targeted drug and to understand at what dose they need to stay under in order to prevent side effects and have a safe dose to use in dogs in future studies. This safe dose would be good for the dogs, for the success of the experiment, and for future human clinical work.

Execution of designed study: The overall problem in the study arose from a discrepancy between batches of compound. Study personnel synthesize their own drugs because they are not commercially available. These drugs have been well tolerated in mice, rats, and the previous dog study. Recently, as study personnel have been preparing for clinical studies, they had a batch synthesized by an outside company. The compound was found to be clean and biologically equivalent to previous batches of drug that was synthesized in the lab. During a study, study personnel were running low on the outside company's manufactured drug and decided to use a new batch of drug that they synthesized within the lab. It appeared to be sufficiently pure for use. However, it was not analyzed until problems began to appear.

2/25/20 - Study personnel started with 200ug/kg of the new batch of drug. Doses were administered between 5:55pm and 5:58pm on three dogs. (b) (6) (Dog 4) was not dosed due to his injury

2/26/20 - The project end points to mimic the action of the free drug was not to reach any MTD. Study personnel had seen a sufficient drop in blood pressure for our study but still had no data on the duration of that effect. From the literature it appeared as though limiting toxicity would be due to a drop in blood pressure and the doses that they were dosing were at the lower end of that change. As such, no adverse events were expected.
Recorded blood pressures were preliminarily analyzed and a minimal drop in blood pressure was observed and the drop was small and so it was impossible to define when the blood pressure returned to normal. Needing this last piece of data in order to understand the drug's duration of effect on the blood pressure, study personnel felt it would be safe to increase the dose again and so the dose was increased to 600ug/kg. Doses were administered late, between 6:29-6:33pm. One dog, (b) (6) received only half of a dose due to him moving a lot during injection.

2/27/20 - Two dogs, (b) (6) are reported to be depressed and (b) (6) vomited. It was reported that they had a "toxic line" and blood work was performed. Bloodwork indicated that there was damage to the kidneys. The animals were admitted to the ICU.

2/28/20 - Study personnel met with PACUC and LAP veterinary staff about the health of the dogs. The consensus was that the dogs are not doing well but that they could be monitored over the weekend to see if the kidney injury was acute and they would quickly recover or whether it would be a long-term problem. Had study personnel known that this would turn out to be the latter, the dogs would have been euthanized right away.

Study personnel re-analyzed the new drug's purity. Though the drug appeared to be good quality, when they analyzed the area under the curve of the drug and compared it to the outside company's standard curve, it was found that there was only 2.9% of the study personnel drug in the sample and the remaining sample was unknown and not detected readily by LCMS. This means that even at 600ug/kg, the dogs were getting only 17.4ug/kg of the in-house drug. That leaves 582.6 ug/kg of other substances, the majority of which was not readily detected on

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LCMS. In previous work, it was demonstrated that the dogs could handle 40ug/kg/day for 19 weeks without apparent toxicity to kidneys or other major organs (determined by blood test and histology). This leads to the conclusion that it was the impurities in the compound that resulted in kidney toxicity. In any future drug formulations, a standard curve of the outside company's drug will serve as a reference standard for formulating any future batches of drug. By comparing the standard curve of the outside company batch of the drug to new batches, study personnel will see if the amount measured is the amount detected and therefore a drug with no impurities.

2/29/20 - Three dogs remained in the ICU at the Purdue Small Animal Hospital.

3/01/20 - Three dogs remained in the ICU at the Purdue Small Animal Hospital.

3/02/20 - Study personnel had another meeting to discuss the project with PACUC and LAP veterinary staff and were informed that the dogs would not be available for any further research while alive. As such, the dogs were scheduled to be euthanized. Due to the meeting finishing after business hours, the dogs could not be removed from the ICU that evening.

3/03/20 - Once the dogs were available for release from the ICU, they were released and euthanized. The telemetry devices were removed, as were the radius and ulna of each dog.

3/04/20 - The dogs were submitted for necropsy at the Animal Disease Diagnostic Lab. Necropsy reports revealed renal and myocardial injury in all three dogs.

PACUC discussed this adverse event and requested that the following additional communication be sent to the PI and study personnel on this protocol:

Three dogs that suffered from renal disease and myocardial injury. The sequence of events that resulted in the ultimate euthanasia of 3 dogs due to non-responsive renal disease was reviewed. The letter the PI wrote to PACUC gave a good summary of what happened during the adverse event, and the committee was satisfied that the injury to the animals was an unfortunate unforeseen occurrence. There are two reasons why the committee was not fully satisfied by the response. First, the letter does not outline what changes will be made going forward to ensure this does not happen again. PACUC is confident that study personnel will analyze future batches of the agent to ensure that it is chemically pure. However, as it does not appear that study personnel evaluated cardiac or renal damage in any other dogs who did not receive the bad batch, there is no way to know what damage, if any, the pure drug is doing in a dog model. The committee would like to see a plan going forward to evaluate kidney and heart damage in dogs receiving the unadulterated agent. The second concern is what appears to be an incidence of noncompliance. It is stated in the protocol that "A member of the protocol will take primary responsibility for monitoring the drug preparation." However, as described in the adverse event letter, the drug was formulated by a remote company and sent to Purdue. Because "monitoring drug preparation" could also be interpreted to mean reconstituting it from a lyophilized state, the committee could not say with assurance that this was an instance of non-compliance. However, the committee asks the PI to clarify this portion of the protocol application.

Regarding the dog that suffered from a thermal burn - A the dog (b) (6) *developed a thermal burn following surgery to implant monitoring equipment. It required extensive treatment*

including debridement and suturing. At the time of the meeting it was reported to be healing via second intention. At the committee meeting, PACUC learned that the dog had been placed on an electric heating device designed for swine. Furthermore, it appeared from the letter and study personnel's plans going forward that you believe that internal body temperature reflects skin temperature and that monitoring body temperature is a way to prevent thermal injury. This is not the case. An electric heating device is not listed in the protocol as a way to maintain body temperature in the dogs on this study. Rather, it is stated in section 3.10 that a warm water blanket, a hot air blanket, and/or an IV fluid warmer would be used. Thus, using an electric heater constitutes noncompliance. The incident that occurred constitutes a major animal welfare issue according to the PACUC Guidelines for resolving issues of non-compliance. Specifically, "performing a procedure that is not addressed in the approved protocol causing animals to endure distress, pain, or suffering" is considered a major issue. The committee would like to see a plan moving forward to ensure that only procedures in the approved protocol occur. The PACUC requires that the use of the heating device that caused the burn on the dog not be used again by the Pre-clinical Research Laboratory no matter what protocol. This will be communicated to that staff directly. The dogs on this protocol can only be warmed using approved devices.

Purdue University would like to reaffirm its commitment to a strong animal care and use program. It is hoped that the actions being taken in response to this incident will serve to strengthen the program of animal care at Purdue University.

Please feel free to contact me if you require additional information.

Sincerely,

(b) (6)

Christopher R. Agnew, Ph.D.
Associate Vice President for Research, Regulatory Affairs

cc: Dr. J. Kritchevsky, Chair, Purdue Animal Care and Use Committee
(b) (6)
Dr. W. Ferner, Director & Attending Veterinarian, Laboratory Animal Program

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