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

April 17, 2019

Re: Animal Welfare Assurance
A4176-01 [OLAW Case U]

(b) (6) MD
CAPT, MC USN
Institute Director
Armed Forces Radiobiology Research Institute
8901 Wisconsin Avenue
Bethesda, MD 20814

Dear CAPT (b) (6),

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your March 29, 2019 letter reporting an instance of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at the Uniformed Services University of the Health Sciences, following up on an initial report on June 4, 2018 and an interim report on August 10, 2018. According to the information provided, OLAW understands that due to a reversal of the cage cards, 48 mice on a radiation study were exposed to the wrong doses of radiation.

The immediate action taken upon discovery consisted of euthanizing 24 mice that received a supra-lethal dose and transferring 24 others to another protocol. The Institutional Animal Care and Use Committee investigated and developed the following corrective actions:

- The standard operating procedure for rodent receipt was modified to address animal receipt and handling, and staff was trained on this.
- Investigators were counseled to ensure experienced staff are involved when conducting animal activities and that the appropriate procedures are conducted.
- Husbandry and research technicians were retrained on identifying common mouse strains and information on strain identification was posted in the housing area.
- Communication between veterinary technical and research staff was improved; the process for requesting mouse identification tattoos has been revised.
- The protocol was amended to adjust the numbers of control and experimental mice.
- No unauthorized costs were charged to the NIH grant and the funding component was notified about the incident.

Based on the information provided, OLAW is satisfied that appropriate actions have been taken to investigate, correct, and prevent recurrence of the noncompliance. We appreciate having been informed about this matter and find no cause for further action by this Office.

Sincerely,

(b) (6)

(b) (6) M.S., D.V.M.
Deputy Director
Office of Laboratory Animal Welfare

cc: IACUC Chair

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
4555 SOUTH PALMER ROAD, BUILDING 42
BETHESDA, MARYLAND 20889-5648
www.usuhs.edu/afrri

29 March 2019

(b) (6), DVM, DACLAM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare (OLAW)
National Institutes of Health (NIH)
Rockledge 1, (b) (6)
6705 Rockledge Drive
Bethesda, MD 20892-7892

Dear Dr. (b) (6)

The Armed Forces Radiobiology Research Institute (AFRRI), in accordance with Animal Welfare Assurance [D16-00610 (A4176-01)] and Public Health Service Policy IV.F.3., provides this report of noncompliance regarding an unintentional deviation that occurred on a Principal Investigator's (PI) animal study protocol, entitled "Evaluation of the IGF-1/Akt/eNOS pathway as a moderator of radiation sensitivity in the laboratory mouse (*Mus musculus*)." This incident was first reported to Dr. (b) (6), OLAW, on 7 June 2018 via e-mail by LTC (b) (6) (b) (6), DVM, MPVM, DACLAM, DACVPM, Veterinary Corps, US Army, Head, Veterinary Sciences Department (VSD), AFRRI.

On the afternoon of 4 June 2018, the PI self-reported to the IACUC Chair, Attending Veterinarian, and IACUC Administrator that the cage cards for two cohorts of different strains of mice were reversed, resulting in the experimental animals being exposed to the wrong doses of radiation on 31 May 2018. The identification mix-up was realized by the research technician and the post-doctoral researcher partway through the day one post-irradiation collection time point. There are different radiosensitivities between mouse strains and although all of the experimental animals were exposed to the wrong dose of radiation, half of the animals (48) remained useful for the study. Twenty-four C3H/HeN mice were euthanized on 1 June since they received a supralethal dose of 9.35 Gray rather than 7.8 Gray. The remaining twenty-four CD2F1 that received 7.0 Gray (sublethal dose) versus the planned 7.8 Gray were not euthanized and subsequently transferred to another protocol. The experiment being conducted was covered under an approved IACUC protocol that is supported by NIH/National Institute of Allergy and Infectious Diseases (NIAID) under a Uniformed Services University of the Health Sciences (USUHS)/AFRRI-NIAID interagency agreement (#AAI17009-001-00001). No unallowable costs were charged against the associated grant and the NIH funding component was notified of the situation.

The IACUC Chair established a subcommittee to further investigate the incident and report their findings to the IACUC for the committee's consideration. Preliminary information of the subcommittee's investigation was shared with IACUC members during the 21 June 2018 and 26 July 2018 meetings. A total of thirteen people were interviewed by the IACUC subcommittee about various processes of animal receipt, identification, transport, and irradiation. All applicable records were inspected and the study protocol was reviewed. It was determined by the IACUC investigative subcommittee that the root cause of the incident was multifactorial. The report was finalized and

accepted at the 13 September 2018 meeting.

The following corrective action plan was implemented after the incident:

1. VSD modified the Rodent Receiving Procedures Standard Operating Procedures (SOP) to reinforce the activities surrounding animal receipt and handling. Training was provided to go over the revisions to the SOP and to highlight the expectations moving forward.

2. PIs and investigative staff were reminded that experienced staff are to be present when conducting experiments using animals. Any procedures that occur must include adequate oversight and experience to verify all animals receive the proper experimental procedures/treatments and are handled properly.

3. Animal care staff and research technicians received training on the identifiable differences between commonly used mouse strains. VSD placed identification documents for common mouse strains within the animal housing areas for immediate reference.

4. The process for PIs to submit technical requests for tattoos has been streamlined by VSD with an emphasis on better lines of communication between the veterinary technical staff and the research staff.

5. The PI determined that the number of control animals originally requested are more than what is needed to repeat the experiment. The PI submitted an amendment to reduce the number of sham animals and to reallocate these animals to the LD70 experimental group.

6. The IACUC recommended that senior leadership determine the financially responsible party (or parties) for any specific losses associated with this incident. Since the PI submitted an amendment to increase the experimental group size for the repeat experiment, and it was approved by the IACUC, sufficient data was collected for analysis and the project has since been completed. The members of senior leadership were in agreement that there was no financial impact to the funding institution, thus determining financial liability became an immaterial matter.

Thank you for your consideration regarding this matter. AFRRI is committed to protecting the welfare of animals used in research and appreciates the guidance and assistance provided by OLAW in this regard. If you require any further information regarding this incident and/or investigation, please contact LTC (b) (6) , Attending Veterinarian, at (b) (6)

(b) (6)

(b) (6) MD
CAPT, MC, USN
Institutional Official
Institute Director, AFRRI

[redacted] (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, April 10, 2019 8:37 AM
To: [redacted] OLAW Division of Compliance Oversight (NIH/OD)
Cc: [redacted] AFRRI-IACUCAdministration
Subject: RE: D16-00610 (A4176-01) Final Report

Thank you for providing this report. We will send an official response soon.

Best regards, [redacted]

[redacted], DVM, DACLAM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

Please note that this message and any of its attachments are intended for the named recipient(s) only and may contain confidential, protected or privileged information that should not be distributed to unauthorized individuals. If you have received this message in error, please contact the sender.

From: [redacted]
Sent: Saturday, March 30, 2019 5:30 PM
To: OLAW Division of Compliance Oversight (NIH/OD) [redacted]
Cc: [redacted] AFRRI-IACUCAdministration <[redacted]>
Subject: D16-00610 (A4176-01) Final Report

Dear OLAW Division of Compliance Oversight,

Attached is AFRRI's final report for the 2018 reportable event.

Kind regards,

[redacted]

A4176

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
4555 SOUTH PALMER ROAD, BUILDING 42
BETHESDA, MARYLAND 20889-5648
www.usuhs.edu/afrri

10 August 2018

MEMORANDUM FOR Chair, IACUC, AFRRI, Bethesda, MD 20889

SUBJECT: IACUC subcommittee's investigative summary surrounding the unintended deviation on protocol 2018-02-001, "Evaluation of the IGF-1/Akt/eNOS pathway as a moderator of radiation sensitivity in the laboratory mouse (*Mus musculus*)"

An IACUC subcommittee was established to investigate and determine the contributing factors that surround the wrongful irradiation of mice belonging to protocol 2018-02-001 and to recommend corrective actions to help avoid future occurrences.

1. **BACKGROUND:** On the afternoon of June 4, 2018, the principal investigator (PI) for protocol 2018-02-001, Dr. (b) (6) officially self-reported that 48 mice (24 C3H/HeN and 24 CD2F1) of the 96 experimental animals were exposed to the wrong dose of radiation on May 31, 2018. This deviation was discovered on June 1st at the first scheduled euthanasia time point, 24 hours post-irradiation. (b) (6) on the protocol, was working with (b) (6) from Dr. (b) (6) laboratory; Dr. (b) (6) is the co-PI on this project. After euthanizing 16 mice in the first cohort, (b) (6) asked (b) (6) whether he had finished working on the C3H/HeN mice and if he was ready to move on to the CD2F1 mice. (b) (6) informed (b) (6) that the mouse strain they were currently working with was CD2F1. It was at this time that they both realized that the strains listed on the identifying cage cards were incorrect, therefore, each strain of mice received the radiation exposure doses intended for the other strain. Although all of the experimental animals were exposed to the wrong dose of radiation, half of the animals (48) remained useful for the study. There are different radiosensitivities between mouse strains; the LD70 for C3H/HeN mice is equivalent to the LD10 for the CD2F1 strain and the LD10 for the CD2F1 corresponded to the LD70 for the C3H/HeN mice so the investigator was able to salvage half of the experiment. Per the recommendation of the contract clinical veterinarian, twenty-four C3H/HeN mice were euthanized on June 1st since they received a supralethal dose of 9.35 Gy rather than 7.8 Gy. The remaining twenty-four CD2F1 that received 7.0 Gy (sublethal dose) versus the planned 7.8 Gy were not euthanized. Additional background information is as follows:

    a. On the afternoon of June 1, 2018, the contract (b) (6) (b) (6) was conducting her weekly vet rounds in the animal vivarium when she noticed that the strain of mice in the cages under protocol 2018-02-001 did not match the stated strain on the cage cards. Upon return to her office, (b) (6) began drafting an email to the PI to notify her of the discrepancy. It was at that time that (b) (6) received a phone call from Dr. (b) (6) describing what had transpired earlier that day (discovery of the strain mistake and incorrect irradiation of the two strains). The PI was advised to euthanize the animals that received the supralethal dose so that they do not experience any unnecessary pain or distress and to also report the incident to the IACUC; the later action was satisfied on June 4th via email with a memorandum to the IACUC Chair.
    b. Since the animals involved are housed at USU's Laboratory Animal Medicine (LAM) vivarium and their IACUC has oversight of the facility, AFRRI's acting IACUC Chair

contacted USU's Attending Veterinarian, LTC [redacted] and IACUC Chair, [redacted] to consult with them for how they would like AFRRI to proceed. LTC [redacted] indicated that USU would like AFRRI's IACUC to conduct the investigation and later inform the USU IACUC of the outcome.

c. On the morning of June 5, 2018, the acting IACUC Chair, LTC [redacted] appointed an investigative subcommittee consisting of MAJ [redacted] and [redacted] to consider the details of the occurrence. Additionally, Dr. [redacted] was instructed not to remove or transfer any animals or adjust any room information as an IACUC investigation would be taking place.

d. The Office of Laboratory Animal Welfare (OLAW) was contacted via email on June 7, 2018 and the PI's self-report memorandum was provided to Dr. [redacted] as a preliminary report for the protocol deviation. OLAW indicated that they will open a new case file and await further information. Shortly thereafter, [redacted] from the Animal Care and Use Review Office (ACURO) was provided the same preliminary information that was provided to OLAW. [redacted] responded with an acknowledgement email and a reminder to provide an update to ACURO following the IACUC's investigation.

e. The AFRRI IACUC was provided Dr. [redacted]'s self-report memorandum and was briefed on the overall occurrence during the June 21, 2018 meeting. Additionally, the preliminary information of the subcommittee's investigation was shared with IACUC members during the June meeting. A more thorough account was given during the July 26, 2018 meeting and a final memorandum of the subcommittee's investigative findings will be provided prior to the next IACUC meeting.

2. **FINDINGS:** Between June 15, 2018 and July 6, 2018, a total of thirteen people were interviewed by the IACUC subcommittee about various processes of animal receipt, identification, transport, and irradiation. All applicable records were inspected and the study protocol was reviewed. It was determined by the IACUC investigative subcommittee that the root cause of the unintentional protocol deviation was multifactorial. The following findings were discovered:

   a. It seems that the most significant contributing factor was the improper labeling of the mouse cages. Based upon the examined process for labeling cages, error would have been significantly reduced if the most experienced husbandry floor leader had provided oversight after animals were placed in their respective cages. This verification typically occurs as part of the receipt and housing process, but in this scenario it did not happen.

   b. Beyond the initial housing of the animals that occurred on May 15, 2018, there were a number of occasions when the animals were either handled by VSD staff or members of the research team prior to the irradiation that occurred on May 31st. There were many missed opportunities both by VSD and the researchers to identify and correct the mistake of the improperly labeled mouse strains such that the wrongful radiation exposures could have been avoided.

   c. Many VSD technicians and animal caretakers are not thoroughly cross-trained to recognize the observable phenotypical differences in less commonly used mouse strains at AFRRI.

   d. This was the first experiment to be executed under the 2018-02-001 protocol. The primary member of the research staff responsible for conducting the procedures was not adequately familiar with the specific animal model chosen nor the designated strains approved for use on the protocol. Overall, the investigative staff was negligent in

providing the proper experience and oversight for the initiation of experiments under a new protocol.

e. Documentation in the animal room was modified by the research staff after the adverse event was reported and these changes were not properly communicated to the IACUC, Attending Veterinarian, or any other VSD staff member. Additionally, animals were moved around into different cage groupings and no one was notified afterwards. This occurred after the PI was specifically told by the IACUC Chair not to make any changes with the animals or the corresponding documentation until further notice as an IACUC investigation was being initiated. The changes that were made by the research staff made it difficult for the IACUC subcommittee to conduct a thorough investigation and be confident in understanding exactly what had transpired from the time the animals arrived at LAM up until the time Dr. (b) (6) reported the incident to the IACUC. Furthermore, information gathered from research staff members during the interviews seemed contradictory to what was either found in email messages or statements made by VSD staff members. Since the cage cards had been altered and the animals moved between cages, it became impossible for the subcommittee to verify information as reported and to have a full understanding of how the process of tattoo identification of the animals may or may not have contributed to the occurrence of the incident.
f. The written communication from the research staff to VSD technicians requesting animals to be tattooed was not adequate in conveying the intended results as later stated by the research staff. Upon reviewing the electronic history for how tattoo requests are provided to VSD, it appears that the VSD technical staff satisfied the request for tattooing according to what was provided by the researchers.
g. The subcommittee observed general indications of poor documentation being practiced by the research staff.

3. **RECOMMENDATIONS:**

a. VSD staff to modify the Rodent Receiving Procedures SOP (VS0204) to reinforce the activities surrounding animal receipt and housing. The changes should include, but are not limited to: 1) having lead husbandry staff (floor leaders) provide direct oversight for all animal receiving events, 2) for VSD to notify research staff that animals have been received, and 3) request that new animals are checked within a set amount of time by research staff and for VSD to be notified via email that the research team has verified their order after the animals have been housed.
b. All investigative staff need to become familiar with all of the animal models they choose before animals are ordered. The burden of responsibility is on the PI to ensure their staff have been appropriately trained and are equipped to successfully carry out their duties prior to the initiation of animal studies. Any training provided to research staff by the PI is expected to be documented and able to be provided upon request.
c. Experienced investigative staff are to be present for the conduction of all animal activities. Any procedures that occur must include adequate oversight and experience to verify all animals receive the proper experimental procedures/treatments.
d. VSD caretakers and technicians are to take documented training led by a qualified individual on the identifiable differences between commonly used mouse strains at AFRRI. VSD has already placed identification documents for common mouse strains within the animal housing areas for immediate reference. Additionally, it is recommended to review gender identification as part of the aforementioned training with the emphasis of verifying gender when housing new animals.

e. Requests for tattooing of animals by VSD staff will be exacting and strain specific. VSD will not carry out any identification procedures if the written documentation is not specific.
f. Recommend that the VSD technician who conducts the tattooing procedure will, in addition to documenting on the tattoo log that the request was fulfilled, send a confirmation email to the PI to notify them that the task has been completed.
g. Investigative staff are to inform VSD staff via email of any change in animal housing groups that differ from how they were initially received and housed.
h. The IACUC Chair to request the AFRRI Directorate to determine the financially responsible party (or parties) for the specific losses associated with this incident.

4. The primary point of contact for this memorandum is MAJ (b) (6)

(b) (6) DVM, DACLAM
Deputy Head, Veterinary Sciences Department
Attending Veterinarian

(b) (6)

(b) (6)

(b) (6)

(b) (6) (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, January 10, 2019 3:20 PM
To: (b) (6) OLAW Division of Compliance Oversight (NIH/OD); (b) (6) (b) (6)
Cc: (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) AFRRI-IACUCAdministration; (b) (6)
Subject: RE: AFRRI INCIDENT REPORT

Thank you for providing the comprehensive interim report. We look forward to receiving the final report.

Best regards, (b) (6)

(b) (6) DVM, DACLAM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: (b) (6)
Sent: Thursday, January 10, 2019 3:07 PM
To: OLAW Division of Compliance Oversight (NIH/OD) (b) (6) (b) (6)
Cc: (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) AFRRI-IACUCAdministration <(b) (6) ; (b) (6) (b) (6)
Subject: RE: AFRRI INCIDENT REPORT

Greetings Dr. (b) (6)

We will promptly provide a final report for this incident. In the meantime, please refer to the attached interim report.

Respectfully,

(b) (6)

(b) (6) (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, January 10, 2019 12:46 PM
To: (b) (6)
Cc: (b) (6) (b) (6) (b) (6) (b) (6); (b) (6) AFRRI-IACUCAdministration
Subject: RE: AFRRI INCIDENT REPORT

Good afternoon,

OLAW doesn't have a record of receiving a final report for this incident. If one is available, please provide it in an email response to this message. If not, please provide an interim report ASAP. Thank you.

Sincerely, (b) (6)

(b) (6) DVM, DACLAM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: (b) (6)
Sent: Thursday, June 07, 2018 3:32 PM
To: OLAW Division of Compliance Oversight (NIH/OD) (b) (6)
Cc: (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) AFRRI-IACUCAdministration < (b) (6)
Subject: AFRRI INCIDENT REPORT

Dr (b) (6)

Please find attached an incident report involving two different mice strains. This was brought to my attention Monday afternoon, with the PI following-up with an incident report. This appears to be a deviation of the protocol and is currently under investigation. Appropriate action will be taken and I will follow-up with an after-action report.

Thank you for your review.

(b) (6) DVM, MPVM, DACLAM, DACVPM
LTC, VC USA
Head, Veterinary Science Department
Armed Forces Radiobiology Research Institute
4555 South Palmer Road, (b) (6)
Bethesda, MD 20889-5648

AA176-U

(b) (6) (NIH/OD) [E]

From: (b) (6) (NIH/OD) [E]
Sent: Thursday, June 07, 2018 4:04 PM
To: OLAW Division of Compliance Oversight (NIH/OD)
Subject: FW: AFRRI INCIDENT REPORT

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, June 07, 2018 4:01 PM
To: (b) (6)
Subject: RE: AFRRI INCIDENT REPORT

Thank you for this prompt preliminary report. We will open a case file and await further information.

Sincerely,(b) (6)

(b) (6), DVM, DACLAM
Acting Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: (b) (6)
Sent: Thursday, June 07, 2018 3:32 PM
To: OLAW Division of Compliance Oversight (NIH/OD)(b) (6)
Cc: (b) (6)(b) (6) (b) (6) (b) (6)(b) (6); (b) (6) (b) (6)AFRRI-IACUCAdministration (b) (6)
Subject: AFRRI INCIDENT REPORT

Dr(b)

Please find attached an incident report involving two different mice strains. This was brought to my attention Monday afternoon, with the PI following-up with an incident report. This appears to be a deviation of the protocol and is currently under investigation. Appropriate action will be taken and I will follow-up with an after-action report.

Thank you for your review.

(b) (6) DVM, MPVM, DACLAM, DACVPM
LTC, VC USA
Head, Veterinary Science Department
Armed Forces Radiobiology Research Institute
4555 South Palmer Road,(b) (6)
Bethesda, MD 20889-5648

(b) (6)

(b) (6)

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
8901 WISCONSIN AVENUE
BETHESDA, MARYLAND 20889-5603

June 4, 2018

MEMORANDUM TO:
LTC (b) (6), DVM, Head, VSD and Chair,
AFRRI IACUC Committee
AFRRI IACUC Committee

SUBJECT: Incident Report on mislabeled mice cages in LAM that lead to delivery of wrong radiation dose.

REFERENCES: (1) Protocol: P-2018-02-001

Purpose. This memo provides the AFRRI IACUC Chair, Attending Veterinarian, and the IACUC Committee with a brief description of a deviation from IACUC approved protocol (P-2018-02-001).

Event summary. This memo reports a deviation from the current protocol regarding the radiation doses to be delivered to the animals.

Event description. On May 31st, a total of 96 mice (48 C3H and 48 CD2F1) were exposed to radiation. Of these, only 48 mice received the correct dose, according to the table below.

Based on the reconstruction of events, the error was due to the fact that upon arrival to LAM, the cards for C3H mice were placed on the CD2F1 cages, and vice-versa. We (my staff and I) were not involved in receiving the mice.

Mice	Dose Expected	Actual Dose Received
24 C3H	7 Gy (LD10)	7.8 Gy (LD70)
24 C3H	7.8 Gy (LD70)	9.35 Gy supralethal
24 CD2F1	7.8 Gy (LD10)	7.0 Gy sublethal
24 CD2F1	9.35 Gy (LD70)	7.8 Gy (LD10)

The discovery of the mistake was done only on June 1st, the first scheduled euthanasia time point (24 hours). On that day, (b) (6) in charge of this project, was working side by side with (b) (6) from Dr (b) (6)'s lab. Dr (b) (6) is the co-PI on this project. After processing the first 16 mice, (b) (6) asked (b) (6) whether he had finished working on the C3H mice and was ready to move to CD2F1. It was at that point that (b) (6) told (b) (6) that the mice strain they were all working on was CD2F1, and they realized that the cage cards were switched on the

cages. To rule out the possibility that a mistake was made at the time of irradiation (when mice were transferred back from the irradiation racks to their home cages), the boxes that were still at LAM and had never been moved to Cobalt were immediately checked. That is when it was discovered that the cage cards were switched on all the mice boxes. This could have happened only at the time of receiving the animals from the vendor.
We have euthanize the 24 C3H mice that received the supralethal dose of 9.35Gy instead of 7.8 Gy, per discussion with (b) (6) since there is no use for those animals. We are waiting to hear back from VSD if the 24 CD2F1 mice that received 7.0 Gy instead of 7.8 Gy will be transferred to VSD training protocol. We request to have 24 C3H and 24 CD2F1 mice credited back to our IACUC protocol.

The entire workplan for this year is 3 iterations of 96 mice each. Half (50%) of our first iteration had to be discarded. The cost of repeating the experiment is not negligible, without accounting for the labor. Eight individuals were involved in the collection of tissues alone. We will draft an estimate of the cost that includes the expenses of the 24 C3H and 24 CD2F1, per diem, tattoo cost, supplies that were used for collecting tissues from mice irradiated with the wrong doses. For each animal, each organ has to be placed in an individual, properly labelled tube. Tissue collection was from 48 mice, 8 organs/mouse (= 384 organs). For each mouse, we used 2 blood collection tubes and we labelled 10 tubes for plasma alone. For each animal, besides collection for molecular analysis, we collect also tissues for histology, requiring samples to be placed in formalin or in cryomolds and OCT. In addition, there is the cost of the van to transport the mice, radiation, and VSD late night checks.

Please do not hesitate to contact me if there are questions

Very Respectfully,

(b) (6)