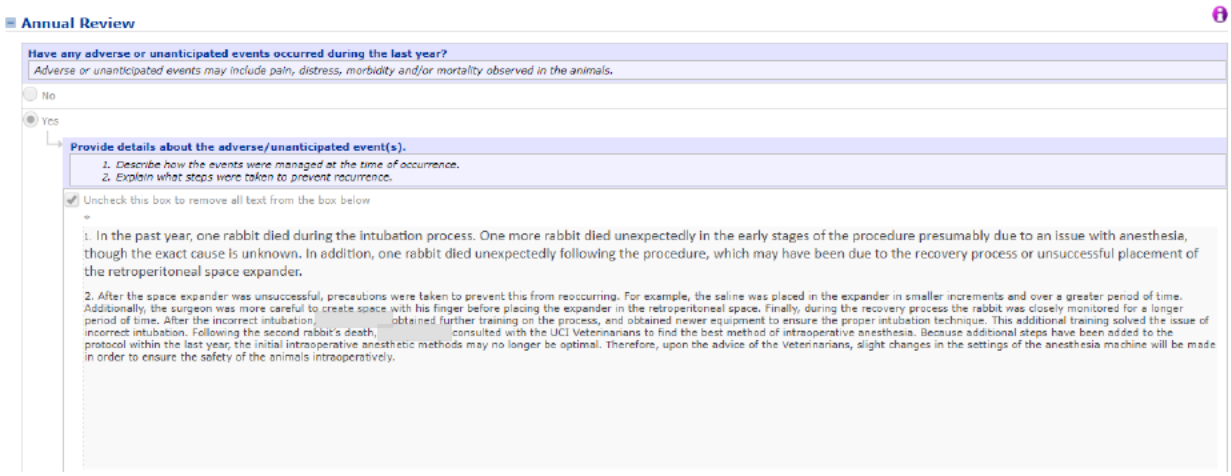
Submitted 11/7/18

Species: Rabbit



Annual Review

Have any adverse or unanticipated events occurred during the last year?

Adverse or unanticipated events may include pain, distress, morbidity and/or mortality observed in the animals.

No

Yes

Provide details about the adverse/unanticipated event(s).

1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

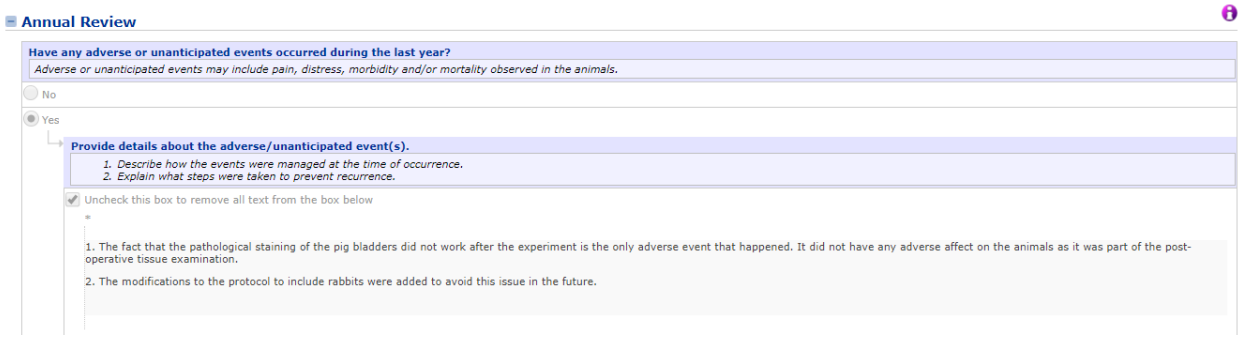
Uncheck this box to remove all text from the box below

1. In the past year, one rabbit died during the intubation process. One more rabbit died unexpectedly in the early stages of the procedure presumably due to an issue with anesthesia, though the exact cause is unknown. In addition, one rabbit died unexpectedly following the procedure, which may have been due to the recovery process or unsuccessful placement of the retroperitoneal space expander.

2. After the space expander was unsuccessful, precautions were taken to prevent this from reoccurring. For example, the saline was placed in the expander in smaller increments and over a greater period of time. Additionally, the surgeon was more careful to create space with his finger before placing the expander in the retroperitoneal space. Finally, during the recovery process the rabbit was closely monitored for a longer period of time. After the incorrect intubation, obtained further training on the process, and obtained newer equipment to ensure the proper intubation technique. This additional training solved the issue of incorrect intubation. Following the second rabbit's death, consulted with the UCI Veterinarians to find the best method of intraoperative anesthesia. Because additional steps have been added to the protocol within the last year, the initial intraoperative anesthetic methods may no longer be optimal. Therefore, upon the advice of the Veterinarians, slight changes in the settings of the anesthesia machine will be made in order to ensure the safety of the animals intraoperatively.

Submitted 11/9/18

Species: Swine, rabbit



Annual Review

Have any adverse or unanticipated events occurred during the last year?

Adverse or unanticipated events may include pain, distress, morbidity and/or mortality observed in the animals.

No

Yes

Provide details about the adverse/unanticipated event(s).

1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

Uncheck this box to remove all text from the box below

1. The fact that the pathological staining of the pig bladders did not work after the experiment is the only adverse event that happened. It did not have any adverse affect on the animals as it was part of the post-operative tissue examination.

2. The modifications to the protocol to include rabbits were added to avoid this issue in the future.

Submitted 10/1/18

Species: Rat

Submitted 11/20/18

Species: Mouse

Submitted 10/26/18

Species: Mouse

Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

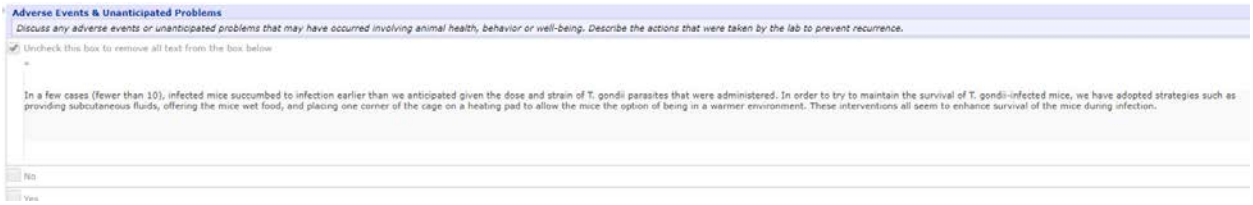
Uncheck this box to remove all text from the box below

In the last 3 years, we had two cased in which developed spontaneous tumors not related to the studies we proposed. Those mice were identified by vivarium staff, which subsequently notified our lab. After examining the mice and considering the recommendation provided by a ULAR veterinarian we decide to humanely euthanize these mice.

Several case (5-7) of mice that developed ulcerative skin lesions due to over grooming or scratching were experienced in the last 3 years. These mice were monitored and cared (trimming of nails and antibiotic ointment treatment) by ULAR veterinarian, and fully recovered.

Submitted 10/3/18

Species: Mouse



Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

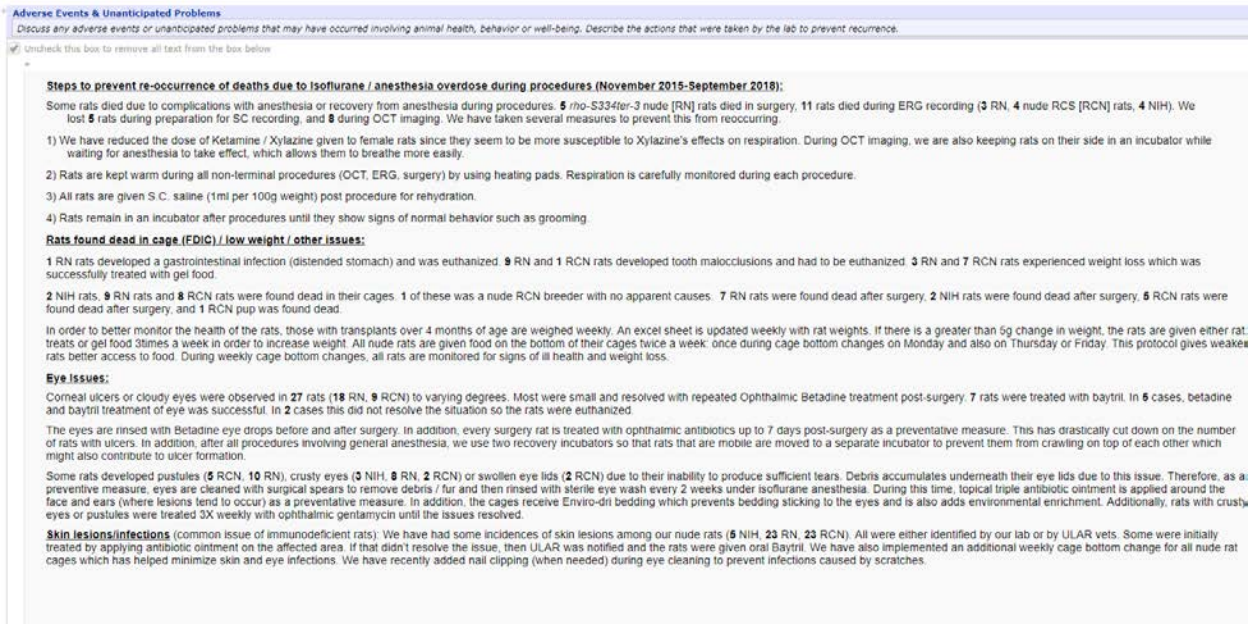
In a few cases (fewer than 10), infected mice succumbed to infection earlier than we anticipated given the dose and strain of T. gondii parasites that were administered. In order to try to maintain the survival of T. gondii-infected mice, we have adopted strategies such as providing subcutaneous fluids, offering the mice wet food, and placing one corner of the cage on a heating pad to allow the mice the option of being in a warmer environment. These interventions all seem to enhance survival of the mice during infection.

No

Yes

Submitted 10/11/18

Species: Mouse, rabbit



Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

Steps to prevent re-occurrence of deaths due to Isoflurane / anesthesia overdose during procedures (November 2015-September 2018):

Some rats died due to complications with anesthesia or recovery from anesthesia during procedures. **5** *rho-S334ter-3* nude [RN] rats died in surgery, **11** rats died during ERG recording (**3** RN, **4** nude RCS [RCN] rats, **4** NIH). We lost **5** rats during preparation for SC recording, and **8** during OCT imaging. We have taken several measures to prevent this from reoccurring.

1) We have reduced the dose of Ketamine / Xylazine given to female rats since they seem to be more susceptible to Xylazine's effects on respiration. During OCT imaging, we are also keeping rats on their side in an incubator while waiting for anesthesia to take effect, which allows them to breathe more easily.

2) Rats are kept warm during all non-terminal procedures (OCT, ERG, surgery) by using heating pads. Respiration is carefully monitored during each procedure.

3) All rats are given S.C. saline (1ml per 100g weight) post procedure for rehydration.

4) Rats remain in an incubator after procedures until they show signs of normal behavior such as grooming.

Rats found dead in cage (FDIC) / low weight / other issues:

1 RN rats developed a gastrointestinal infection (distended stomach) and was euthanized. **9** RN and **1** RCN rats developed tooth malocclusions and had to be euthanized. **3** RN and **7** RCN rats experienced weight loss which was successfully treated with gel food.

2 NIH rats, **9** RN rats and **8** RCN rats were found dead in their cages. **1** of these was a nude RCN breeder with no apparent causes. **7** RN rats were found dead after surgery, **2** NIH rats were found dead after surgery, **5** RCN rats were found dead after surgery, and **1** RCN pup was found dead.

In order to better monitor the health of the rats, those with transplants over 4 months of age are weighed weekly. An excel sheet is updated weekly with rat weights. If there is a greater than 5g change in weight, the rats are given either rat treats or gel food 3times a week in order to increase weight. All nude rats are given food on the bottom of their cages twice a week: once during cage bottom changes on Monday and also on Thursday or Friday. This protocol gives weaker rats better access to food. During weekly cage bottom changes, all rats are monitored for signs of ill health and weight loss.

Eye Issues:

Corneal ulcers or cloudy eyes were observed in **27** rats (**18** RN, **9** RCN) to varying degrees. Most were small and resolved with repeated Ophthalmic Betadine treatment post-surgery. **7** rats were treated with baytril. In **5** cases, betadine and baytril treatment of eye was successful. In **2** cases this did not resolve the situation so the rats were euthanized.

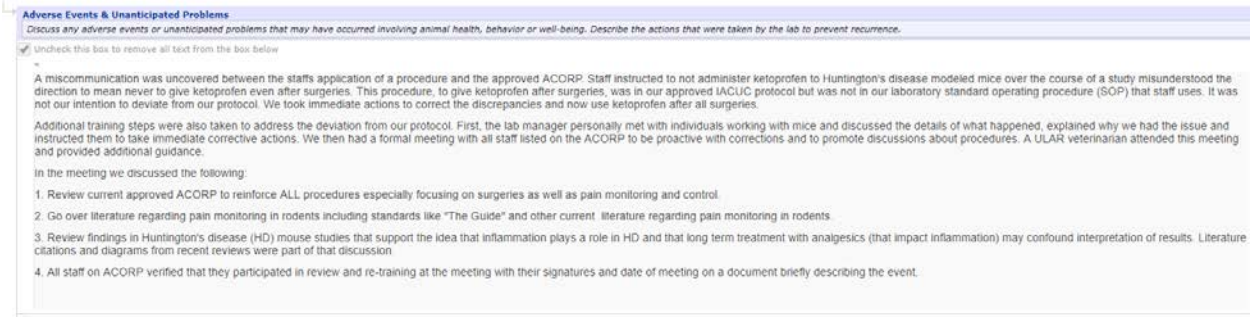
The eyes are rinsed with Betadine eye drops before and after surgery. In addition, every surgery rat is treated with ophthalmic antibiotics up to 7 days post-surgery as a preventative measure. This has drastically cut down on the number of rats with ulcers. In addition, after all procedures involving general anesthesia, we use two recovery incubators so that rats that are mobile are moved to a separate incubator to prevent them from crawling on top of each other which might also contribute to ulcer formation.

Some rats developed pustules (**5** RCN, **10** RN), crusty eyes (**3** NIH, **8** RN, **2** RCN) or swollen eye lids (**2** RCN) due to their inability to produce sufficient tears. Debris accumulates underneath their eye lids due to this issue. Therefore, as a preventive measure, eyes are cleaned with surgical spears to remove debris / fur and then rinsed with sterile eye wash every 2 weeks under isoflurane anesthesia. During this time, topical triple antibiotic ointment is applied around the face and ears (where lesions tend to occur) as a preventative measure. In addition, the cages receive Enviro-dri bedding which prevents bedding sticking to the eyes and is also adds environmental enrichment. Additionally, rats with crushed eyes or pustules were treated 3X weekly with ophthalmic gentamycin until the issues resolved.

Skin lesions/infections (common issue of immunodeficient rats): We have had some incidences of skin lesions among our nude rats (**5** NIH, **23** RN, **23** RCN). All were either identified by our lab or by ULAR vets. Some were initially treated by applying antibiotic ointment on the affected area. If that didn't resolve the issue, then ULAR was notified and the rats were given oral Baytril. We have also implemented an additional weekly cage bottom change for all nude rat cages which has helped minimize skin and eye infections. We have recently added nail clipping (when needed) during eye cleaning to prevent infections caused by scratches.

Submitted 10/26/18

Species: Mouse



Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

A miscommunication was uncovered between the staffs application of a procedure and the approved ACORP. Staff instructed to not administer ketoprofen to Huntington's disease modeled mice over the course of a study misunderstood the direction to mean never to give ketoprofen even after surgeries. This procedure, to give ketoprofen after surgeries, was in our approved IACUC protocol but was not in our laboratory standard operating procedure (SOP) that staff uses. It was not our intention to deviate from our protocol. We took immediate actions to correct the discrepancies and now use ketoprofen after all surgeries.

Additional training steps were also taken to address the deviation from our protocol. First, the lab manager personally met with individuals working with mice and discussed the details of what happened, explained why we had the issue and instructed them to take immediate corrective actions. We then had a formal meeting with all staff listed on the ACORP to be proactive with corrections and to promote discussions about procedures. A ULAR veterinarian attended this meeting and provided additional guidance.

In the meeting we discussed the following:

1. Review current approved ACORP to reinforce ALL procedures especially focusing on surgeries as well as pain monitoring and control.

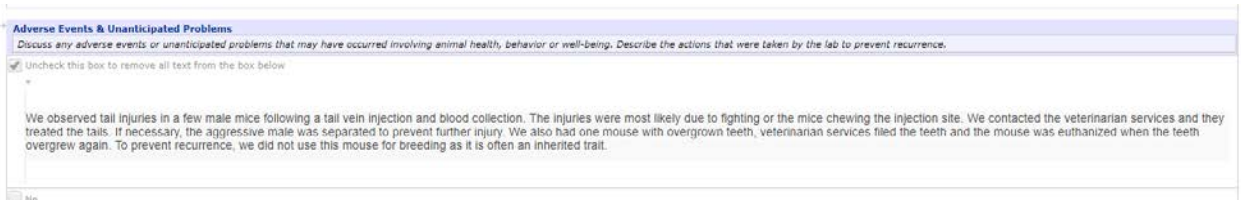
2. Go over literature regarding pain monitoring in rodents including standards like "The Guide" and other current literature regarding pain monitoring in rodents.

3. Review findings in Huntington's disease (HD) mouse studies that support the idea that inflammation plays a role in HD and that long term treatment with analgesics (that impact inflammation) may confound interpretation of results. Literature citations and diagrams from recent reviews were part of that discussion.

4. All staff on ACORP verified that they participated in review and re-training at the meeting with their signatures and date of meeting on a document briefly describing the event.

Submitted 11/14/18

Species: Mouse



Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

We observed tail injuries in a few male mice following a tail vein injection and blood collection. The injuries were most likely due to fighting or the mice chewing the injection site. We contacted the veterinarian services and they treated the tails. If necessary, the aggressive male was separated to prevent further injury. We also had one mouse with overgrown teeth, veterinarian services filed the teeth and the mouse was euthanized when the teeth overgrew again. To prevent recurrence, we did not use this mouse for breeding as it is often an inherited trait.

Submitted 12/17/18

Species: Mouse, Rat

Adverse Events & Unanticipated Problems

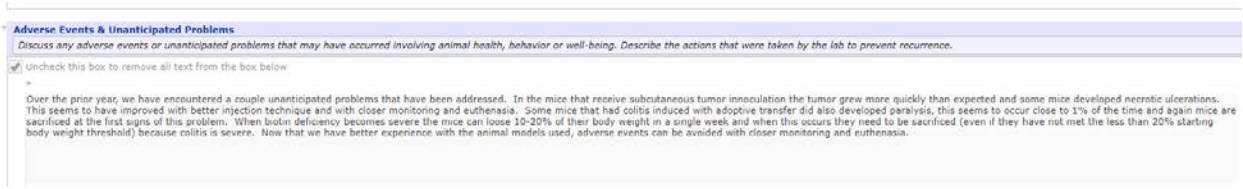
Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

We proposed to use Rotenone to induce a Parkinson's disease (PD) like phenotype in rats, and we followed what has been published in the literature. However rotenone injected rats showed poor health and were euthanized. If we repeat that experiment we will decrease the rotenone dose considerably or change it for another PD inducing agent.

Submitted 12/17/18

Species: Mouse



Adverse Events & Unanticipated Problems

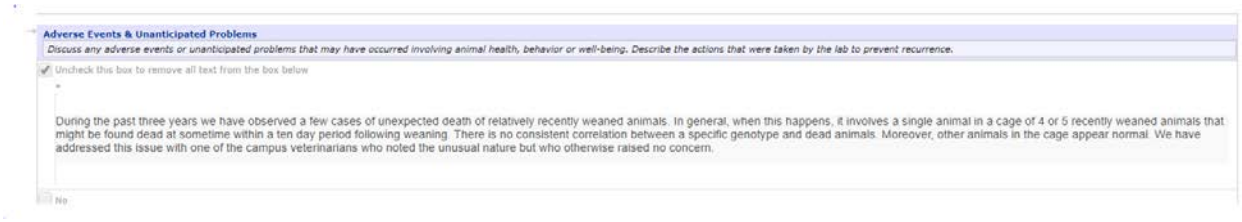
Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

Over the prior year, we have encountered a couple unanticipated problems that have been addressed. In the mice that receive subcutaneous tumor innoculation the tumor grew more quickly than expected and some mice developed necrotic ulcerations. This seems to have improved with better injection technique and with closer monitoring and euthenasia. Some mice that had colitis induced with adoptive transfer did also developed paralysis, this seems to occur close to 1% of the time and again mice are sacrificed at the first signs of this problem. When biotin deficiency becomes severe the mice can loose 10-20% of their body weight in a single week and when this occurs they need to be sacrificed (even if they have not met the less than 20% starting body weight threshold) because colitis is severe. Now that we have better experience with the animal models used, adverse events can be avoided with closer monitoring and euthenasia.

Submitted 1/10/19

Species: Mouse



Adverse Events & Unanticipated Problems

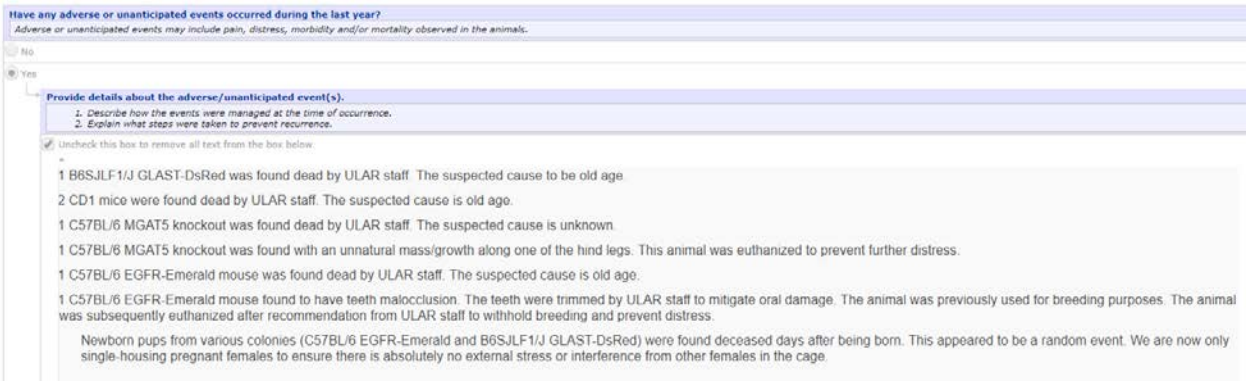
Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

☑ Uncheck this box to remove all text from the box below

During the past three years we have observed a few cases of unexpected death of relatively recently weaned animals. In general, when this happens, it involves a single animal in a cage of 4 or 5 recently weaned animals that might be found dead at sometime within a ten day period following weaning. There is no consistent correlation between a specific genotype and dead animals. Moreover, other animals in the cage appear normal. We have addressed this issue with one of the campus veterinarians who noted the unusual nature but who otherwise raised no concern.

☐ No

February 9, 2019



Have any adverse or unanticipated events occurred during the last year?

Adverse or unanticipated events may include pain, distress, morbidity and/or mortality observed in the animals.

No

Yes

Provide details about the adverse/unanticipated event(s).

1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

Uncheck this box to remove all text from the box below.

1 B6SJLF1/J GLAST-DsRed was found dead by ULAR staff. The suspected cause to be old age.

2 CD1 mice were found dead by ULAR staff. The suspected cause is old age.

1 C57BL/6 MGAT5 knockout was found dead by ULAR staff. The suspected cause is unknown.

1 C57BL/6 MGAT5 knockout was found with an unnatural mass/growth along one of the hind legs. This animal was euthanized to prevent further distress.

1 C57BL/6 EGFR-Emerald mouse was found dead by ULAR staff. The suspected cause is old age.

1 C57BL/6 EGFR-Emerald mouse found to have teeth malocclusion. The teeth were trimmed by ULAR staff to mitigate oral damage. The animal was previously used for breeding purposes. The animal was subsequently euthanized after recommendation from ULAR staff to withhold breeding and prevent distress.

Newborn pups from various colonies (C57BL/6 EGFR-Emerald and B6SJLF1/J GLAST-DsRed) were found deceased days after being born. This appeared to be a random event. We are now only single-housing pregnant females to ensure there is absolutely no external stress or interference from other females in the cage.

May 11, 2019

Did any adverse or unanticipated events with animal health, behavior or well-being occur during the last year?

Adverse events or unanticipated problems may include pain, distress, morbidity and/or mortality observed in the animals.

No

Yes

Provide details about the adverse/unanticipated event(s), including:

1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

We noticed during the past year one project (9 out of a Total 84 Rag1 Immunodeficient Mice), which had a higher infection rate at the site of incision than previous projects. In this case 4 mice (5% of total) developed a lump at their infection site. For the first animal, at the time of dissection, we alerted UCI Vet staff who sent samples for testing, which were positive for Staph infection. Previously we have noted this problem only rarely, in a large group of animals (Less than 1%). Interestingly, we saw similar growths on the lip of an additional 5 mice (6% of total) from the study, which were not part of our surgical procedure. To prevent recurrence for future projects, we have reviewed our surgical aseptic technique with UCI Vets, and have implemented several recommended changes.

We have since since similar growths on lips and in abdominal/genital region in additional immunodeficient animals, before we do any surgical procedures, and in some cases in breeding animals. We are considering putting all immunodeficient animals on prophylactic antibiotic feed.

May 10, 2019

Did any adverse or unanticipated events with animal health, behavior or well-being occur during the last year?

Adverse events or unanticipated problems may include pain, distress, morbidity and/or mortality observed in the animals.

○ No

◉ Yes

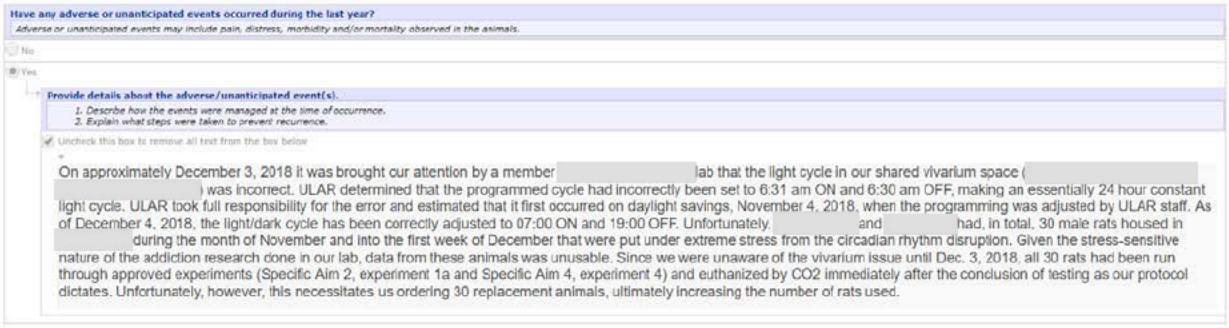
Provide details about the adverse/unanticipated event(s), including:
1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

Two mice died after application of oxymetazoline (Rhofade). After consultation with the company (Allergan), we estimated that the quantity we applied was too high. We reduced the quantity and did not experience any further unanticipated event.

[redacted]

[redacted]

February 8, 2019



Have any adverse or unanticipated events occurred during the last year?

Adverse or unanticipated events may include pain, distress, morbidity and/or mortality observed in the animals.

No

Yes

Provide details about the adverse/unanticipated event(s).

1. Describe how the events were managed at the time of occurrence.
2. Explain what steps were taken to prevent recurrence.

Uncheck this box to remove all text from the box below

On approximately December 3, 2018 it was brought our attention by a member [redacted] lab that the light cycle in our shared vivarium space ([redacted]) was incorrect. ULAR determined that the programmed cycle had incorrectly been set to 6:31 am ON and 6:30 am OFF, making an essentially 24 hour constant light cycle. ULAR took full responsibility for the error and estimated that it first occurred on daylight savings, November 4, 2018, when the programming was adjusted by ULAR staff. As of December 4, 2018, the light/dark cycle has been correctly adjusted to 07:00 ON and 19:00 OFF. Unfortunately [redacted] and [redacted] had, in total, 30 male rats housed in [redacted] during the month of November and into the first week of December that were put under extreme stress from the circadian rhythm disruption. Given the stress-sensitive nature of the addiction research done in our lab, data from these animals was unusable. Since we were unaware of the vivarium issue until Dec. 3, 2018, all 30 rats had been run through approved experiments (Specific Aim 2, experiment 1a and Specific Aim 4, experiment 4) and euthanized by CO_2 immediately after the conclusion of testing as our protocol dictates. Unfortunately, however, this necessitates us ordering 30 replacement animals, ultimately increasing the number of rats used.

April 30, 2019

Did any adverse or unanticipated events with animal health, behavior or well-being occur during the last year?

Adverse events or unanticipated problems may include pain, distress, morbidity and/or mortality observed in the animals.

○ No

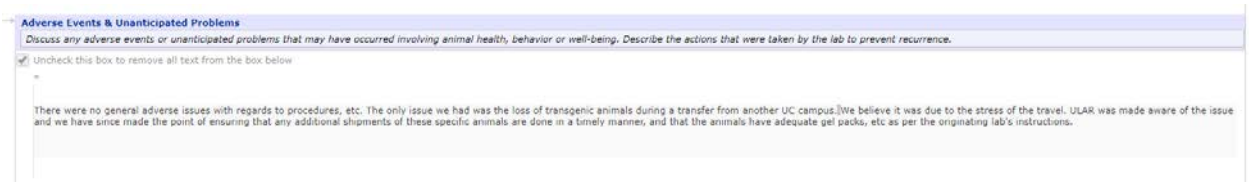
◉ Yes

Provide details about the adverse/unanticipated event(s), including:
1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

1. C57Bl6 mice that were ordered came with wounds on the back and were not able to be used for implantation studies. Extra mice were ordered to anticipate adverse events and those animals. Animals were normal otherwise.

2. C57Bl6 mice have not been ordered since then, but individual caging and communication with the vendor could prevent this.

February 14, 2019



Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

There were no general adverse issues with regards to procedures, etc. The only issue we had was the loss of transgenic animals during a transfer from another UC campus. We believe it was due to the stress of the travel. ULAR was made aware of the issue and we have since made the point of ensuring that any additional shipments of these specific animals are done in a timely manner, and that the animals have adequate gel packs, etc as per the originating lab's instructions.

March 13, 2019

Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

Uncheck this box to remove all text from the box below

There have been several instances of overcrowding in our colony, but this problem has improved with increased attention of students and staff. The older K14DN-Clim mice sometimes develop large skin wounds and need to be euthanized. Depending on our past experiences, the mice under imiquimod treatment would show dehydration leading to weight loss and decreased activity levels, we injected PBS to relieve the dehydration and it surely worked.

February 25, 2019

Adverse Events & Unanticipated Problems

Discuss any adverse events or unanticipated problems that may have occurred involving animal health, behavior or well-being. Describe the actions that were taken by the lab to prevent recurrence.

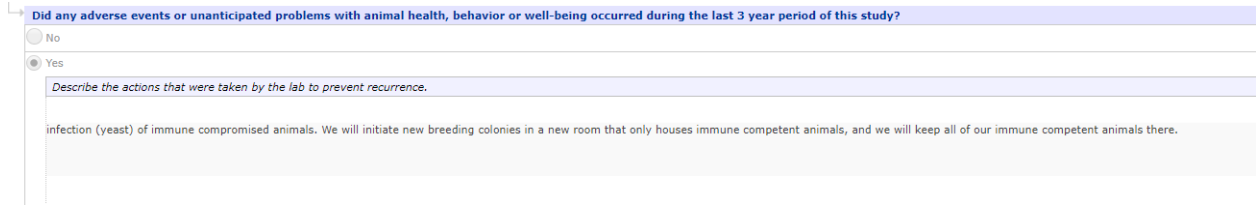
Uncheck this box to remove all text from the box below

There were 23 unexpected rat deaths.

There were 15 rats that died in the Nicotinamide Mononucleotide project after 5/6 nephrectomy surgery; following discussion with ULAR vets, adjustments were made to the post-surgical protocol including a prophylactic dose of antibiotic and enhanced aseptic technique.

The other 8 rats died in the JZL184 project. 2 normal rats died before surgery without any intervention, two more died within 48 hours after surgery and 4 rats died during PET/CT scanning. We immediately notified veterinarian and ULAR staff, and checked with the supplier to see if the animals may have had pre-existing health issues. There was concern for excessive urination in the normal animals, however monitoring of blood glucose, body weight and daily water intake did not show any diabetes mellitus. We could not identify the cause of the early deaths. No unexpected losses occurred with subsequent surgeries.

April 11, 2019



Species: Mouse

Submitted 6/7/19

Annual Review

Have any adverse or unanticipated events occurred during the last year?

Adverse or unanticipated events may include pain, distress, morbidity and/or mortality observed in the animals.

No

Yes

[IACUC-ADMIN USE ONLY] -- Follow-up by the IACUC office (if applicable):

Provide details about the adverse/unanticipated event(s).

1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

Uncheck this box to remove all text from the box below

1. With mice receiving the rhofade application (which is a FDA-approved topical compound), we have experienced problems with mortality. Multiple animals have died before reaching day 7, and the application region has dried out.

2. We currently are trying to understand what the cause is of the mortality, to see if this is anecdotal or is due specifically to rhofade application. We added one day post surgery before applying the rhofade, to provide the animal with an extra day to recover from the surgery. If we observe another early death, we will need to discuss a potential protocol deviation with the contract sponsor (Allergan) and then request a protocol modification for IACUC review.

Species: Mouse

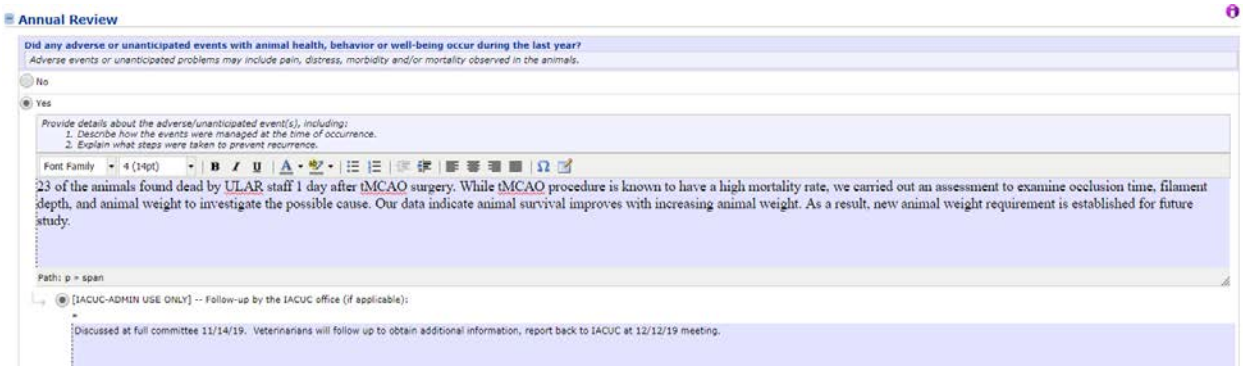
Submitted 6/19/19

Species: Peromyscus

AR submitted to IACUC 5/24/19

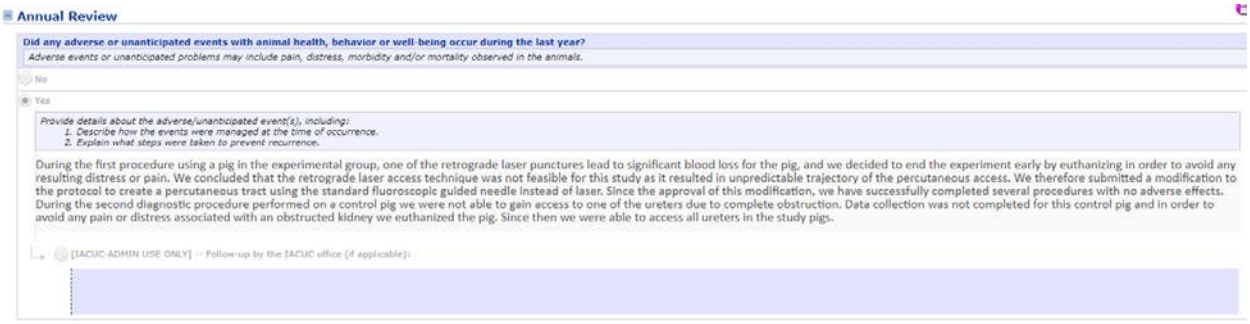
Species: Mouse

Submitted 8/26/19



Species: Swine

Report submitted 8/29/19



Annual Review

Did any adverse or unanticipated events with animal health, behavior or well-being occur during the last year?

Adverse events or unanticipated problems may include pain, distress, morbidity and/or mortality observed in the animals.

No

Yes

Provide details about the adverse/unanticipated event(s), including:
1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

During the first procedure using a pig in the experimental group, one of the retrograde laser punctures lead to significant blood loss for the pig, and we decided to end the experiment early by euthanizing in order to avoid any resulting distress or pain. We concluded that the retrograde laser access technique was not feasible for this study as it resulted in unpredictable trajectory of the percutaneous access. We therefore submitted a modification to the protocol to create a percutaneous tract using the standard fluoroscopic guided needle instead of laser. Since the approval of this modification, we have successfully completed several procedures with no adverse effects. During the second diagnostic procedure performed on a control pig we were not able to gain access to one of the ureters due to complete obstruction. Data collection was not completed for this control pig and in order to avoid any pain or distress associated with an obstructed kidney we euthanized the pig. Since then we were able to access all ureters in the study pigs.

[IACUC ADMIN USE ONLY] -- Follow-up by the IACUC office (if applicable):

Submitted 8/21/19 – Compliance resolved and report approved 10/18/19

Annual Review

Did any adverse or unanticipated events with animal health, behavior or well-being occur during the last year?

Adverse events or unanticipated problems may include pain, distress, morbidity and/or mortality observed in the animals.

○ No

◉ Yes

Provide details about the adverse/unanticipated event(s), including:
1. *Describe how the events were managed at the time of occurrence.*
2. *Explain what steps were taken to prevent recurrence.*

There were a total of 108 animals that manifested some level of complication associated with the experimental procedures during the last year. 17 had treatable skin lesions and 48 had treatable eye issues; these animals recovered and were returned to the study (65 animals, representing 60% of the total complications).

The remaining 43 rats either died during procedures or were euthanized:

- 18 rats were unable to recover from anesthesia (15 during surgical procedures, 3 during OCT imaging). To address this issue and prevent recurrence, all laboratory personnel have been retrained and anesthetized animals are now monitored more frequently (approximately every 2 minutes) during anesthesia.
- 2 animals were lost during testing of different dosages of immunosuppressants; we have adjusted our dosages accordingly.
- 2 animals were lost during a routine blood collection procedure. To prevent recurrence, we now use disposable rat-restraining cones to calm and restrain animals for this procedure.
- 5 animals were lost due to infections, cancer, and other complications common to immunodeficient animals. Another 15 animals were found dead of unknown causes. We have increased the frequency of monitoring of all our rats, and seek guidance from ULAR veterinarians whenever possible.
- 1 rat was accidentally left in a procedure room incubator following an OCT imaging procedure on a Friday (7/26/19) and was found dead the following Monday (7/29/19). We are working with the IACUC to address this non-compliance.