PURDUE UNIVERSITY

PURDUE ANIMAL CARE AND USE COMMITTEE

September 2012

PACUC Newsletter

Visit the PACUC website at www.purdue.edu/animals for forms, instructions, policies and guidelines.

Inside this issue:

PACUC Orientation Program for Vertebrate Animal Users

An orientation program for **new** faculty, staff, and students, who will be using vertebrate animals in research, teaching, and/or testing, will be held on the following date during the fall semester:

Tuesday, September 4, 2012, 1:30-3:00 p.m. in LILY 1-117.

Attendance at this session is mandatory for personnel (i.e., faculty, staff, students) who wish to initiate work with vertebrate animals at Purdue University. Personnel will not be approved to work with animals until such time that they have attended the orientation program or completed the program on-line. We strongly encourage participating in an "in-person" program; however, it may be completed on-line if your schedule does not allow for in-person participation. Please go to the following URL:
http://www.purdue.edu/research/vpr/rschadmin/rschoversight/animals/onlineorientation.php
The password to enter is "pass" (without the quotation marks).

This program presented by staff of the Purdue Animal Care and Use Committee (PACUC) and the Laboratory Animal Program (LAP) is designed to introduce you to the Purdue system for maintaining regulatory compliance with federal and University guidelines and ensuring humane care and use of animals.

Registration is required to attend the September 4 orientation program and may be done via e-mail to Lisa Snider, PACUC Administrator, at ldsnider@purdue.edu. Your name, department, and e-mail address are necessary for registration.

Implementation of Coeus Electronic Software

As of July 9, 2012, Coeus has been implemented university-wide in areas using vertebrate animals for research, teaching, and testing. These areas are now submitting all PACUC-related items (protocols, amendments, etc.) electronically. The PACUC office is no longer accepting any paper copies.

Below are helpful links to get started. You can log in using your Purdue career account and password.

CoeusLite Login Page: https://coeus.itap.purdue.edu/coeus/userAuthAction.do

Investigator Quick Reference Guides: http://www.purdue.edu/business/coeus/IACUC_Protocols/Quick_Reference_IACUC/index.html

Some important things that you should know:

1. When completing a new application, you must complete #1, Data Field Tabs and the questionnaire which is item #2 on the electronic system. Completion of this questionnaire is required.
2. You must complete and upload the PACUC Protocol Application Attachment in the Attachment Uploads area of the electronic system. The attachment can be found on the PACUC website, http://www.purdue.edu/research/vpr/rschadmin/rschoversight/animals/forms.php. The long version of the protocol application may no longer be used.
3. When completing an Amendment using Coeus, you must upload the current Amendment form located on the above PACUC website.
4. ALWAYS USE THE MOST UPDATED PACUC FORMS LOCATED ON THE WEBSITE, DO NOT USE FORMS THAT YOU HAVE SAVED ONTO YOUR COMPUTERS.
5. Contact coeushelp@purdue.edu if you require any assistance. Someone will be available to assist you immediately.

Lisa Snider
PACUC Administrator

Mouse Quarantine

All mice entering a Purdue University animal facility from non-approved sources will be required to enter quarantine until the animal health status of the animals can be verified. This requirement is for facilities participating in the centralized management effort (currently BIOL, ANSC, NS, and PHAR).

Non –approved sources would include collaborating universities, colleges or other institutions that may wish to donate mice to an investigator at Purdue University. Approved sources would include commercial vendors such as Harlan, Taconic, Charles River and Jackson Laboratories. Mice from approved sources would not be required to go through the quarantine process.

- Sentinel serology and parasitology information is required for the past year for mice coming from non-approved sources. This information should include tests for the same pathogens and parasites excluded from Purdue Universities animal facilities. A Purdue University Laboratory Animal Program Veterinarian will review that information and approve/disapprove the shipment.
- Mice from non-approved sources will be housed in RHPH quarantine.
- Options for confirming the health of the mice are as follows:
    1. An extra mouse sent with the incoming shipment will be sampled for serology, para sitology and a necropsy performed six weeks after arrival.
    2. A percentage of the mice sent will be live bled for serology and samples taken for parasitology six weeks after arrival.
    3. Sentinel mice will be exposed to dirty bedding from the incoming mice. After six weeks of exposure the sentinel mice will be sampled for serology, parasitology and a necropsy performed.
    4. Rederivation and subsequent serology, parasitology and necropsy on foster moms after any pups are weaned.
- Samples for serology will be sent to IDEXX – RADIL or Charles River.
- Mice will be release from quarantine pending approval of all test results by a Laboratory Animal Program Veterinarian.
- All costs for shipping animals, per diems while in quarantine and serologic testing will be the responsibility of the Purdue University principle investigator importing the mice.
- All Purdue Animal Care and Use Committee (PACUC) requirements, i.e., an approved PACUC protocol and Animal Requisition Form must be on file with the PACUC office prior to shipment of any animals.

Important Message from The PACUC Office

As we begin a new academic year, with new students, staff and programs, I wanted to highlight a few important issues regarding utilization of vertebrate animals in research, teaching, and testing activities. Purdue policy and federal regulations require that, prior to any use of vertebrate animals in research, teaching, or testing, a protocol describing that use must be reviewed and approved by the Purdue Animal Care and Use Committee (PACUC). A critical component of PACUC's responsibility is the documentation that all individuals who will participate in the activity involving vertebrate animals have received appropriate training to ensure that they are qualified for their role in the project.

There are several important actions necessary for those investigators with active, approved protocols for use of animals, and new postdoctoral, student, or technical staff joining their research, teaching, or testing projects. First, before new personnel work with vertebrate animals at Purdue University, they must complete an orientation session provided by the PACUC and Purdue Laboratory Animal Program (LAP). Second, before new personnel begin independent work on a project they must receive training appropriate to their role. During this period of training, the individual may work with animals only under direct supervision by a qualified person. Finally, when training is complete and the individual is ready to begin independent work under the approved protocol, the individual's qualifications must be documented to PACUC through submission of an animal use qualification form, and the new personnel must be formally added to the project through an amendment to the protocol.

Please note that requirements for PACUC/LAP orientation, providing and documenting project-specific training, and adding personnel to protocols apply to any and all individuals who will work directly with or care for vertebrate animals at Purdue, regardless of whether this activity is short term or long term. Thus, the requirements apply equally to temporary postdoctoral associates, students, and technical staff working on projects, or graduate students experiencing laboratory rotations before selecting an advisor, as they do to full time, permanent staff.

All information necessary to submit or amend a protocol, or to document qualifications, are available on the PACUC/LAP website [www.purdue.edu/animals]. If you have any questions regarding these requirements or wish assistance with training, protocols, or documenting qualifications, do not hesitate to contact the PACUC/LAP office at 494-9163.

Best wishes for a rewarding and productive academic year!

Lisa Snider, CPIA

PACUC Administrator

From the Transgenic Mouse Core Facility (TMCF)

Are Your Mice Safe?

What if there was a fire? Or a flood? Or hepatitis swept through your mouse facility? What if you lost all your mice? *What would it cost to start your mouse colony from scratch?*

Preservation of mouse lines through **cryopreservation** is the best safeguard against any type of disaster. The Transgenic Mouse Core Facility (TMCF) can freeze and store both sperm and embryos. And we maintain duplicate cryobanks in 2 different locations for extra security.

For $1200 ($300 for PUCCR members) and 8 male mice, we will collect, freeze and store 300 of your precious embryos. We will superovulate wild type females in our barrier facility, mate them with your male mice, collect the fertilized eggs, freeze them 25-30/straw in a controlled-rate freezer and store them in liquid nitrogen. Typically, 4 rounds of freezing are necessary to generate 300 embryos.

Or for $530 ($110 for PUCCR members) and 2 male mice, we will collect, freeze and store 20 aliquots of your precious sperm. Males will be sacrificed, their sperm collected in cryoprotectant medium, frozen 10ul/straw and stored in liquid nitrogen.

Frozen embryos can be recovered by simply thawing them and transferring them into surrogate mothers, while frozen sperm must be thawed and used in an *in vitro* fertilization (IVF) procedure to generate fertilized eggs. These embryos are then transferred into surrogate mothers and all pups born are transferred to your facility at weaning.

But, that's not all we do. TMCF also offers several other services that can benefit your research.

What if you are already infected with a pathogen? Or maybe you need to bring an infected line of mice into a clean mouse facility? We can clean up your mice by **re-derivation** through embryo transfer. All we need are 2 males of breeding age. We will superovulate wild type females in our barrier facility, bring them to your facility to mate with your "dirty" boys and then collect the fertilized eggs. The eggs will be washed and transferred into clean surrogate mothers in our barrier facility. When the pups are weaned, we will send serum from the mothers for serological testing in accordance with your animal facility's requirements. And once the pups are confirmed clean they will be transferred to you.

What about problem breeders? Is he the last of his kind and just not breeding anymore? Did you forget him on the shelf and suddenly realize he is the only one left and really old? Don't panic. We also offer **IVF** for these situations. We will harvest sperm from your precious mouse and perform IVF with superovulated wild type female mice. Fertilized eggs will be transferred into surrogate mothers and you will get all the pups when they are weaned.

Or maybe you need 100 mice of the same age all at the same time and are daunted by the logistics and space constraints of setting up such a large colony for a single breeding. We can help you here too with our **Speed Expansion** service. Using IVF along with embryo transfer, and just 1 or 2 of your males, we can generate large numbers of mice of the same age and genotype.

Are you tired of the sky-high prices the commercial companies charge for mice? Or just in awe of the ever-increasing shipping charges? Take heart. We also offer **C57BL/6NHsd mice for sale** from our production colony. Supply is limited, but they are only $5/weanling and no shipping! Compare that to Harlan or JAX!

Here's a little something for those of you who want to take your mouse into the tissue culture lab. We can make you a line of **mouse embryonic fibroblasts** (MEFs) from the mouse of your choice. We just need 1 pregnant mouse and we'll give you enough MEFs to last a lifetime!

And last, but by no means least, our **brand new Genotyping Service** is underway!! Don't waste time and money re-inventing the genotyping wheel with every new student. Let us do your genotyping for you. Just give us your tail tips and we'll digest them, extract the DNA, do the PCR and send you the results within 2 days. All for just $4.50/tail! It couldn't be easier.

And a final word to those of you making genetically modified mice – Don't worry, we haven't lost sight of our primary focus. The Transgenic Mouse Core Facility is still making **transgenic and knock-out mice**!

So, whatever your mouse problem, give us a call at 49-63352 and see how we can help. (Unless your mouse is sick, then you should call your vet.)

For prices and details on these and our other services, see the TMCF website - http://www.bio.purdue.edu/research/groups/tmcf/

Upcoming Workshops!

I will be offering the following training workshops with a maximum of **5** participants in each session. (If you have any questions or special requests, please do not hesitate to contact me; 494-2521)

These hands-on workshops are designed to introduce the participant to the basic techniques in the laboratory rat and mouse. The Handling/Restraint workshop is a prerequisite for participation in injection, oral gavage, blood collection, and catheter placement workshops; unless participant has had previous training and/or experience in this area. A minimum of 3 days notice is requested for cancellation.

Workshop dates are filled on a first-come, first-serve basis.

Location for the following workshops – Meet in AHF 1155.

If you are interested in participating in a workshop, please complete the enrollment form indicating which date you would like to attend, or contact Carol Dowell at dowellc@purdue.edu or 494-2521. (If the following times do not fit your schedule or training needs, I would be happy to set up training for most any species on an individual basis.)

Registration form: Attached to the email that brought you the PACUC Newsletter

- *Basics of Rodent Handling, Restraint, and Normal Behavior.*
  - 8/24/12 – Friday 8:30 – 10:30am
  - 8/28/12 – Tuesday 8:30 – 10:30am
  - 8/29/12 - Wednesday 1:30 – 3:30pm
  - 9/13/12 – Thursday 9:00 – 11:00am
  - 9/27/12 – Thursday 9:00 – 11:00am
  - 10/12/12 – Friday 8:30 – 10:30am
- *Injections in the rat and mouse; ID, IV, IM, SC, IP*
  - 9/5/12 - Wednesday 8:30 – 10:30am
  - 9/14/12 - Friday 9:00 – 11:00am
  - 10/11/12 – Thursday 9:00 – 11:00am
- *Blood Collection in the Rat and Mouse*
  - 9/6/12 - Thursday 8:30 – 10:30am
  - 9/17/12 - Monday 1:30 – 3:30pm
  - 10/10/12 - Wednesday 9:00 – 11:00am

- *Tail Vein Injection and Catheter Placement in the Lab. Rat and Mouse*
  - 9/19/12 - Wednesday 8:30 – 10:30am
  - 10/1/12 - Monday 1:30 – 3:30pm

- *Rodent Oral Gavage.*
  - 8/31/12 – Friday 9:00 – 10:15am
  - 9/18/12 - Tuesday 9:00 – 10:15am

- *Isoflurane Gas Anesthesia*
  - 9/13/12 - Thursday 1:30 – 3:00pm
  - 9/20/12 - Thursday 8:30 – 10:00am

- *Wound closure and Suturing Basics*
  - 9/12/12 - Wednesday 9:00 – 10:30am
  - 9/26/12 - Wednesday 1:30 – 3:00pm

- *Aseptic Technique / Surgical Preparation*
  - 9/11/12 – Tuesday 8:30 – 10:00am
  - 9/25/12 – Tuesday 8:30 – 10:00am

- *Euthanasia / Basic Necropsy / Organ Identification*
  - 9/7/12 - Friday 1:30 – 2:45pm
  - 9/26/12 - Wednesday 8:30 – 9:45am

Combined Techniques * (Prior experience/training in ALL courses is required)

This workshop is designed to incorporate all that you have learned regarding rodent handling, injections, blood collection, anesthesia, surgical prep, wound closure and euthanasia.

10/17/12 – Wednesday 8:30 – 11:30am

10/18/12 – Thursday 8:30 – 11: 30am

Carbon Dioxide (CO_2) Euthanasia of Rodents

Despite its widespread use, rodent euthanasia methods using CO_2 are not standardized.

The PACUC does have Guidelines for the Use of $CO_2$for Euthanasia of Rodents (see: http://www.purdue.edu/research/vpr/rschadmin/rschoversight/animals/policies.php)

Pertinent points in the current PACUC guidelines that need to be followed include:

- Euthanasia chambers should be free of debris and excreta and cleaned after each use.
- Chambers should not be crowded and allow for normal postural movements of the animals. Essentially this means the euthanasia chamber should be large enough to permit each animal to stand with all four feet on the floor of the chamber and have sufficient space to turn around and perform normal postural adjustments. Following the space recommendations in the Guide for the Care and Use of Laboratory Animals would be most appropriate.
- Incompatible animals or unfamiliar animals should not be mixed in the chamber.
- Pre-filling of the chamber is not recommended as it has been shown that high concentrations of CO_2 are distressful to some animals.
- CO_2 should be introduced into the chamber at a rate of 10-20% of the chamber volume per minute to reduce distress to the animal(s). For most systems currently in use at Purdue this means an introduction gas flow rate that is barely audible to the individual adding CO_2 to the euthanasia chamber. Once the animal(s) appear unconscious flow rate can be increased.
- Death of the animal(s) must be verified prior to disposal. Use of a secondary means (e.g., creating a pneumothorax or performing cervical dislocation) is preferred

The current PACUC Guidelines reference the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia that were published in June 2007. In 2011 the AVMA revised those guidelines and these revised guidelines are planned for publication this fall (2012). Once published, Purdue will be required to adhere to these published guidelines.

The PACUC is anticipating a need to alter its recommendations for carbon dioxide euthanasia of rodents once the AVMA guidelines are formally published. Anticipated changes include:

1. Excess gas must be allowed to escape from the chamber in a way that allows a gradual increase in the concentration of CO_2 at the floor of the container that holds the animal. Escape of the gas mixture through a port, or other opening at the top of the chamber, must occur in a controlled manner that neither pressurizes the chamber nor permits reflux of room air into the chamber.
2. Chambers will need to be filled with CO_2 at a flow rate that balances the time to unconsciousness and avoids aversive stimuli such as noise or high velocity air movement. To accomplish this CO_2 will need to be delivered using a two-stage regulator, with the second stage capable of adjustable fixed flow rates. I.e., attachment of a flow meter to the CO_2 regulator apparatus.
3. Changes in the animal's environment or novel conditions should be minimized to the degree that is practical. Rodents are sensitive to their environment and to handling. Removal from the home cage, regrouping with other animals, introduction to new sites and odors, and transport and placement into the euthanasia chamber can alter physiologic and metabolic parameters and possibly cause stress. Performing euthanasia in the home cage, using carts that are quiet, roll freely, and do not jostle cages or occupants, and minimizing regrouping to prevent social aggression are simple approaches to lessening potentially stressful conditions.

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PURDUE ANIMAL CARE AND USE COMMITTEE

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Carbon Dioxide (CO_2) Euthanasia of Rodents (continued from page 8)

Implementing these items will require setting up systems with appropriate lids for chambers/home cages and addition of a flow meter to the system and using a predetermined appropriate flow rate for the specific sized chamber/cage. It is anticipated that the upgrade of each CO_2 system, to include those maintained in investigators laboratories, will cost ~$ 600 to $800 per system.

The PACUC will keep staff informed of needed changes once the AVMA recommendations are finalized.

PACUC Meeting Dates—Fall 2012

Meeting Date	Deadline for Protocol Submission
September 19	August 29
October 17	September 26
November 14	October 24
December 19	November 28