



# ANIMAL TRACKS



A newsletter for the Duke research community

October 2007

<http://vetmed.duhs.duke.edu>

## No space in the mouse nursery (Problems with Mouse Breeding Colony Density)

Ever been on a trip and you can't find a place to stay? Tried to attend a conference and got turned away because the room was full? Why? Overcrowding – too many people wanting to stay at the same place at the same time. Local governments and fire departments place limits on how dense humans can be in a given space, usually for reasons of safety or sanitation. There are also limits on the density of animals in the same cage – same issue, and really for the same reason.

The Duke animal care program receives significant funding resources from the NIH and other federal government agencies. As such, we are required to abide by the various federal polices concerning animal care, including the NIH/NRC Guide for the Care & Use of Laboratory Animals. A particular area of confusion has been mouse pup management. Let's look at the issues of 'how many is too many,' 'when must I count them,' and 'when must I separate them.' The goal of this discussion is to keep your laboratory fully compliant with federal rules and regulations.

Our animal program uses mouse cages that range from 67 - 75 square inches of floor surface, but the majority of mouse cages are 75 square inches in size. For simplicity, this review will use a 75 square inch cage. The recommendations discussed below are based upon existing NIH/PHS/OLAW policy and The Guide.

**Post-Weaning Animals:** According to The Guide, mice of weaning age and above, must be housed at a minimum density of 15 square inches per mouse. Using a 75 square inch cage, and 15 square inches per mouse, that means that no more than 5 post-weanling mice may be in the cage at the same time (without an IACUC 'EXEMPTION'). Numbers of post-weanling animals, rather than body weight (the old method of determining cage density), is the basis for assessing overcrowding.

**Weaning:** Mice should be weaned by 21 days of age. For certain genetically engineered mice (GEM), weaning may need to be delayed until 28 or even 35 days of age. Delaying weaning beyond 21 days of age requires an 'EXEMPTION TO POLICY.' The exemption is approved by the IACUC upon provision of sufficient scientific justification. The method of obtaining an 'EXEMPTION' involves submission of an amendment form (available on the animal program web site).

See MOUSE BREEDING ... Page 6

## Keeping Labs Smoke-Free (October 7—13: Fire Prevention Week)



On an early February morning in 2002, an Environmental Services employee smelled something burning in one of the labs where she was working. When she unlocked the lab door, she saw flames coming from a piece of rubber tubing that supplied natural gas to a Bunsen burner – the flames were burning through the workbench. It was determined the small pilot light on the burner had been left on overnight, starting the fire.

One year ago another early morning fire, started by a faulty power strip, caused extensive damage to a Duke research lab. In addition to losing data, equipment and lab materials, the presence of chemicals and a small stock of radioactive materials created concern among the first responders who extinguished the fire, as well as the surrounding labs and community, about potential contamination. The fire resulted in an extensive, costly clean-up operation.

Fires can happen anytime, anywhere, and they do, sometimes despite our best efforts to be safe. However, many fires happen with our help, and could have been prevented with appropriate fire safety practices. Labs, with their numerous heat sources and storage of combustible, flammable, and hazardous materials and compressed gases, have the potential to be dangerous places and require diligence to keep all people and property safe.

Some fire safety tips for labs include:

- \* Clear clutter, like paper, boxes and other combustible materials, when working with heat sources (such as Bunsen burners) at your bench.
- \* Do not overload electrical outlets and keep electrical equipment stored away from water sources.
- \* Store flammable materials in approved cabinets, away from heat or spark sources.
- \* Do not store flammables near oxidizing agents.

See FIRE PREVENTION AT DUKE ... page 5

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# Canine Trick or Treat!

(We Prefer Bones, Please)



## New BMBL edition issued

In February 2007, the US Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health published the 5th edition of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). The BMBL provides guidelines applicable to both *in vitro* and *in vivo* work with human and zoonotic pathogens.

The BMBL includes ‘agent summary statements’ that describe the appropriate biosafety levels for working with various microorganisms, as well as recommendations for proper handling, safety equipment, and facility safeguards that should be in place when conducting research on particular agents. Agent summary statements are included for microorganisms that fit the following criteria:

- ⇒ “the agent is a proven hazard to laboratory personnel working with infectious materials;
- ⇒ “the agent has a high potential for causing [laboratory-associated infections] even though no documented cases exist;
- ⇒ “the agent causes grave disease or presents a significant public health hazard<sup>1</sup>.”

Updates since the last edition include the addition of new or updated agent summary statements for those agents now classified as ‘Select Agents’, including an updated Agent Summary Statement for influenza viruses, and a new chapter on biosecurity, defined as “the discipline addressing the security of microbiological agents and toxins and the threats posed to human and animal health, the environment, and the economy by deliberate misuse or release<sup>1</sup>.”

1. DHHS, CDC & NIH. *Biosafety in Microbiological and Biomedical Laboratories*, 5th edn. (US Government Printing Office, Washington, DC, 2007). <http://www.cdc.gov/OD/OHS/biosafety/bmb15/mb15toc.htm>.

## Clearance vs. Approval

(Learning to Speak the IACUC’s language)

You have submitted your protocol (or amendment). It is being reviewed ... and then you get an Email from OESO telling you that your protocol (or amendment) has received ‘CLEARANCE.’ This is good news! You can start the animal studies .... Or can you?

The truth is NO! An OESO or EOHW ‘Clearance’ IS NOT the same as an IACUC approval. Your protocol MUST receive IACUC approval prior to initiating any animal activity, including ordering animals.

The Duke animal program is an integrated campus-wide program. This means that the IACUC is only one part of the process that must be completed prior to initiating animal studies. To facilitate the review process for PIs, the IACUC coordinates protocol review with OESO and EOHW. Sometimes this can be confusing, so let’s take a moment and consider the review and approval process for animal use applications at Duke.

**STEP 1:** You submit an animal use application to the IACUC by sending it via Email to IACUC@duke.edu, by Faxing it to the IACUC at 668.6725, or by hand carrying it to the IACUC Office in the Hock Plaza Building.

**STEP 2:** A Grants and Contracts Specialist (Jenny Baker or Sandra Pierce) will do an administrative review, confirming protocol administrative information has been provided and is accurate, and send a copy of the protocol (or amendment) to OESO and EOHW for their review.

**STEP 3:** A veterinarian with the Office of Animal Welfare Assurance performs a Veterinary Pre-Review, assuring the animal welfare and well being issues have been addressed, and where possible making recommendations on other protocol methods and proposals that will enhance the protocol and increase the opportunity for IACUC approval.

**STEP 4:** OAWA receives OESO and EOHW clearance, attaching all necessary documents to the master file and assigns an IACUC Reviewer for the protocol.

**STEP 5:** The IACUC Reviewer (who is an IACUC member) will perform a review and may ask additional clarification questions (in addition to the Veterinary Pre-Review).

**STEP 6:** The IACUC Reviewer presents the protocol to the Committee. The IACUC votes on the disposition—the options are Approve As Is; Deferred for Designated Member Review; Deferred for Full Committee Review; Tabled for a re-write; or Disapprove).

**STEP 7:** The OAWA prepares the necessary correspondence and advises the PI of the Committee’s decision. If either OESO or EOHW have not provided a ‘CLEARANCE’ for the protocol, then the approval letter cannot be given to the PI. **Duke policy prohibits the IACUC release of an approval until ‘CLEARANCE’ has been received by OESO and EOHW.**



## REGULATORY WATCH

(Q & A From THE NIH)

*Question: May investigators use non-pharmaceutical grade compounds in animals?*

**NIH/OLAW's Answer:** OLAW and USDA consider that the use of non-pharmaceutical grade compounds should be based on:

- \* scientific necessity;
- \* no availability of an acceptable veterinary or human pharmaceutical-grade compound; and
- \* specific review and approval by the IACUC.

Investigators and IACUCs should consider relevant animal welfare and scientific issues including safety, efficacy, and the inadvertent introduction of new variables. Cost savings alone do not adequately justify the use of non-pharmaceutical-grade compounds in animals. Although the potential animal welfare consequences of complications are less evident in non-survival studies, the scientific issues remain the same and the principles and need for professional judgment outlined above still apply.

*Question: Are major multiple survival surgical procedures permitted on a single animal?*

**NIH/OLAW's Answer:** The [Guide](#) discourages multiple major survival surgical procedures on a single animal but provides that if scientifically justified by the user and approved by the IACUC they may be permitted. [Guide](#) examples of justifications include (1) procedures that are related components of a research project; (2) conservation of scarce animal resources, and (3) clinical necessity. The [Guide](#) does not consider cost savings alone an adequate reason for performing major multiple survival surgical procedures.

Note that under the AWA regulations, major multiple survival surgical procedures are permitted only if the multiple procedures are included within one proposal, justified for scientific reasons, and approved by the IACUC. The AWA requires prior authorization of the USDA Administrator, Animal and Plant Health Inspection Service, for an exemption from this requirement. [See 9 CFR, Part 2, Section 2.31 (d)(1)(x)]

*Question: May the investigator begin animal work before receiving IACUC approval?*

**NIH/OLAW's Answer:** No.

*Question: What guidelines should IACUCs follow for fishes, amphibians, reptiles, birds, and other nontraditional species used in research?*

**NIH/OLAW's Answer:** PHS Policy is intentionally broad in scope and does not prescribe specifics about the care and use of any species, assigning that task to the IACUC and allowing for professional judgment. Many of the principles embodied in [The Guide](#) can generally be adapted to the care and use of various kinds of nontraditional research animals. IACUCs may seek the advice of experts when necessary, and refer to scientific-based publications prepared by professional organizations with interest in various species. Appendix A of [The Guide](#) references many such publications.

*Question: Does the PHS Policy apply to use of animal tissue or materials obtained from dead animals?*

**NIH/OLAW's Answer:** The use of dead animals or parts of animals is not covered by the PHS Policy unless the activity involves (1) killing animals for the purpose of obtaining or using their tissues or other materials, or (2) project-specific ante mortem manipulation of animals prior to killing them. If either circumstance is applicable to the acquisition of dead animals, body parts or tissues, prior IACUC protocol review and approval are required.

*Question: Does the PHS Policy apply to larval forms of amphibians and fish?*

**NIH/OLAW's Answer:** Yes, larval forms of fish and amphibians have vertebrae and are covered by the PHS Policy.

# What Investigators Need to Know About the Use of Animals

(Department of Health and Human Services, National Institutes of Health)

“Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative.” *U.S. Government Principle IV, 1985*

Principal Investigators are responsible for the scientific and technical aspects of a grant award and must ensure compliance with Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals when using live, vertebrate animals. PHS Policy incorporates U.S. Government Principles, the Guide for the Care and Use of Laboratory Animals, and the Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia. Vertebrate animals include traditional laboratory animals, farm animals, wildlife, and aquatic animals. Animal use encompasses research, teaching, or testing. Generation of custom antibodies is considered an activity involving vertebrate animals.

**Who Must Comply With the PHS Policy?** The PHS Policy on Humane Care and Use of Laboratory Animals applies to extramural and intramural activities supported by any PHS agency, including the National Institutes of Health (NIH), the Food and Drug Administration, and the Centers for Disease Control and Prevention. All funding mechanisms, including research and training grants, cooperative agreements, and contracts, conducted at domestic and foreign institutions, are covered by the Policy.

**What Is an IACUC?** Institutional Animal Care and Use Committees (IACUCs) are local institutional committees with federally mandated oversight responsibilities, including:

- ◆ Reviewing animal-use protocols;
- ◆ Reviewing significant changes to protocols;
- ◆ Evaluating institutional compliance with PHS Policy, U.S. Department of Agriculture (USDA) Animal Welfare Regulations, and institutional policies;
- ◆ Monitoring institutional animal care and use programs, including inspecting animal facilities;
- ◆ Reviewing concerns about animal care or use; and
- ◆ Reporting noncompliance and suspensions to the Office of Laboratory Animal Welfare (OLAW).

## Institutional and Investigator Responsibilities:

1. Describing proposed use of animals in grant applications.
2. Obtaining IACUC approval prior to using animals and prior to implementing significant changes.
3. Ensuring research is conducted in accord with the protocol.
4. Complying with institutional policies and procedures.
5. Addressing significant changes to the use of animals in progress reports.
6. Addressing changes in the use of animals that may be a potential change in scope (A change in scope is a change in the direction, type of research, aims, objectives, or purpose

of the project).

**Applying for Funding:** The proposed involvement of vertebrate animals is evaluated as part of the agency peer review process. In addition to providing IACUC approval status, applicants must address five points in the Research Plan of the grant application:

- ◇ A detailed description of the proposed use of the animals, including species, strains, ages, sex, and numbers.
- ◇ Justification of the use of animals, choice of species, and numbers to be used.
- ◇ Information on the veterinary care of the animals.
- ◇ A description of the procedures for ensuring humane treatment (i.e., minimization of discomfort, distress, pain, and injury).
- ◇ The method of euthanasia, the reasons for its selection, and consistency with the AVMA Euthanasia Report.

Failure to address these elements will result in the application being designated incomplete and is grounds for PHS to defer the application or may negatively affect the priority score.



“Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals...” *U.S. Government Principle VIII, 1985*

**Obtaining IACUC Review:** IACUC approval is required prior to award except in rare circumstances.

Your IACUC will require you to submit information about the care and use of animals on a protocol form. Most animal-use protocols require a description of the following elements:

- \* Research project;
- \* Rationale for animal use and consideration of alternatives;
- \* Justification for the choice of species and number of animals;
- \* Research procedures involving animals;
- \* Procedures to minimize pain and distress;
- \* Animal living conditions and veterinary care;
- \* Names and qualifications of personnel who will perform work with animals;
- \* Method of euthanasia; and
- \* Endpoint criteria.

The use of animals as described in the protocol approved by the IACUC must be congruent with the description in a competing grant application. Any modification required by the IACUC that affects the content of the application must be submitted to the agency along with the IACUC approval date.

*Continued on the next page ...*

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**Receiving an Award:** To receive an award the grantee organization and every performance site where animal work will be performed must have an Animal Welfare Assurance approved by OLAW. OLAW will contact an organization with specific instructions when an Assurance is required. An inter-institutional Assurance is negotiated when the grantee does not have its own animal facilities and the animal work will be performed at an institution with an Assurance.

Foreign institutions provide a Statement of Compliance with Standards for Humane Care and Use of Animals.

SBIR/STTR investigators should be aware of the requirements in order to address them in a timely fashion so that the necessary Assurances are in place and grants can be awarded.

The date of IACUC approval is essential in order to receive an award and will be requested if not previously provided.

**Post Award:** IACUC approval is required at least every 3 years (annually if covered by USDA regulations). Significant changes in animal care and use are to be approved by the IACUC prior to implementation. Check with your IACUC to determine what constitutes a significant change.

Conducting research in the absence of a valid IACUC approval or implementing a significant change without IACUC approval constitutes noncompliance.

Investigators also must be aware of and comply with additional institutional policies that may be more restrictive.

*Visit the following Web sites for additional information:*

**NIH Office of Laboratory Animal Welfare**

<http://grants.nih.gov/grants/olaw/olaw.htm>

**1996 Guide for the Care and Use of Laboratory Animals**

<http://www.nap.edu/readingroom/books/labrats/>

**NIH Grants Policy Statement (2003)**

[http://grants.nih.gov/grants/policy/nihgps\\_2003/index.htm](http://grants.nih.gov/grants/policy/nihgps_2003/index.htm)

**PHS 398 Grant Application**

<http://grants.nih.gov/grants/funding/phs398/phs398.html>

**SF424 (R&R) Application and Electronic Submission Information**

<http://grants.nih.gov/grants/funding/424/index.htm>

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**

The National Institutes of Health (NIH), part of the Department of Health and Human Services, is the principal health research agency of the U.S. Federal Government. The Office of Extramural Research (OER) provides policies and guidelines for extramural research grants administration. OER has primary responsibility for developing and implementing NIH Grants Policy, including policies related to data and safety monitoring; protection of human subjects; humane use and care of laboratory animals; program guidelines; invention reporting requirements; and the information systems for grants administration. Within OER, the Office of Laboratory Animal Welfare has primary responsibility for animal welfare policy matters.



From Page 1 ... FIRE PREVENTION AT DUKE

It's also important to know what to do if a fire does break out in your lab:

- \* Know the location of the pull stations and fire extinguishers.
- \* Know at least two ways out of your work area in case of an emergency evacuation. Make sure neither way out involves an elevator – always use the stairs in an emergency.
- \* Know where your Emergency Assembly Point (meeting place) is for your lab. This information can be found on the OESO Fire Safety Web site, under the "[Site Specific Fire Plans](http://www.safety.duke.edu/FireSafety/SSFP/Default.asp)" link. (<http://www.safety.duke.edu/FireSafety/SSFP/Default.asp>)

The National Fire Protection Association (NFPA) sponsors Fire Prevention Week (FPW) each October, focusing on a different theme each year and various fire hazards and prevention techniques for the home, school and workplace. **This year's FPW theme is "Practice Your Escape Plan" and takes place October 07 – 13.** A room can go up in flames in a few minutes, so it's important to practice fire drills both at home and at work.

To commemorate FPW, there will be a number of activities taking place around the Duke community in October, including:

- \* "How to Cook and Grill Safely" demonstrations by the Grilling Store (October 16, 9am and 11am, MSRB Quad)
- \* Laboratory Natural Gas Safety by PSNC Energy (October 24, 12pm, 147 Nan Duke)
- \* Fire truck and Sparky visits to Children's Health Center and Children's Campus
- \* Fire safety information booths
- \* Hands-on fire extinguisher training
- \* Drills around Duke.

Look for more details and information on these activities on OESO's [Fire Safety Web site](http://www.safety.duke.edu/FireSafety/Default.asp) (<http://www.safety.duke.edu/FireSafety/Default.asp>). For more information on FPW and fire safety resources, you can look at the [NFPA Web site](http://www.nfpa.org/) (<http://www.nfpa.org/>).



**From Page 1: MOUSE BREEDING**

**Active Breeding mice:** When discussing breeding animals, the issues of proper cage density are not entirely intuitive. Below are specific guidelines, that when followed, meet the federal requirements, and assure animal welfare and well being. These guidelines include:

- \* No more than three adults in a cage when a litter is born or when there are more than five pups older than seven days of age. This means that one male and two females (triad mating) is possible, but
- \* No more than two adults and ten pups in the cage when any of the pups are older than seven days of age. More than ten pups older than seven days can reside in a cage with one adult female if the pups are the progeny of the adult female.
- \* No litters in the same cage that are more than two weeks apart in age.
- \* Pups must be weaned at 21 days of age unless an exemption has been approved by the IACUC or the DLAR veterinary staff for health concerns.

**IMPORTANT NOTE:** Breeding cages containing pups past the age of weaning, where some combination of breeding adults and post-weanling age offspring are in the cage at the same time, **DO NOT** meet the required density guidelines and will result in a non-compliance for overcrowding.

**SUGGESTIONS TOWARD SUCCESSFUL BREEDING MANAGEMENT:** There are certain breeding practices that are a hallmark of a successful laboratory. These include:

- \* Writing the date of birth of all litters and weaning date on the cage card will help with multiple litters in the room at the same time.
- \* Monitoring animal housing density one to two times a week will catch those animals approaching their weaning date and allow sufficient time to schedule a weaning activity with laboratory members.
- \* If weaning is missed, and DLAR has identified overcrowded cages, then respond immediately to DLAR's notification. The laboratory staff has 24 hours to correct the overcrowded condition before it becomes a non-compliance. Checking cages one to two times a week will prevent this situation from occurring!
- \* Remove the male from harems after breeding is confirmed! Removing the male will prevent post-partum breeding, which is the primary cause of multiple ages of litters from the same female in the same cage. Removing males will decrease the risk of cannibalism after birth of the offspring, which sometimes occurs with aggressive males.

While the responsibility of preventing overcrowding is a laboratory responsibility, DLAR will assist research staff in achieving the goal. When DLAR identifies an overcrowded cage, it means the laboratory has failed in its obligation to prevent overcrowding! Research staff should not depend upon DLAR for identifying cages that need to be weaned. To help with identifying problem areas, DLAR will:

- \* Observe cages daily during normal husbandry procedures.
- \* Mark overcrowded cages and notify the Principal Investigator by Email or fax when observing an overcrowded cage.
- \* DLAR will identify as overcrowded, the following situations:
  - \* pups are more than 21 days of age (unless there is an IACUC or DLAR - approved exemption in weaning age and it is designated on the breeding card); or
  - \* litters are more than 14 days apart in age; or
  - \* if there are more than 10 pups and 2 adults in the cage and at least one litter is 7 days old; or
  - \* if there are more than 5 mice of past weaning age in the cage.

**Overcrowding is a serious issue and must be corrected!** If the research laboratory does not correct the condition within 24 hours after notification:

- \* DLAR staff will separate the animals.
- \* DLAR will charge the Principal Investigator a 'service fee' for each cage of animals generated.
- \* If there is no space in which to separate the animals, DLAR will make one additional attempt to contact the PI. If the PI does not respond within an additional 24 hours, DLAR will euthanize animals to correct the cage density. The PI will be charged a 'service fee' for correcting the overcrowding situation.

**Exemptions:** In certain cases, and for the well being of the young mice, weaning may need to be delayed until 28 days or even later. Since this is a deviation from the NIH guidelines, the IACUC must grant an 'EXEMPTION' for non-routine management of mice. The method to obtain an exemption is to file an 'AMENDMENT FORM' (available on the web site). With sufficient scientific or welfare justification, the IACUC may grant an exemption and the young mice may be retained with the dam for the time required to assure healthy and vigorous mice.

## Upcoming Dates & Deadlines

October 4	Significant Change Meeting
October 8	New Protocol Deadline
October 8	Significant Change Deadline
October 18	Significant Change Meeting
October 25	New Protocol Meeting
October 29	Significant Change Deadline
October 29	New Protocol Deadline
November 8	Significant Change Meeting
November 15	New Protocol Meeting

**Deadlines are 5 PM on the date listed! No exceptions!**