



ANIMAL TRACKS

A newsletter for the Duke research community



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

YOUNG SCIENTISTS IN THE LAB? TAKE NOTE ...

Tara Romano
Education & Training Coordinator, OESO

Summer is a great time for potential young scientists to gain valuable experience working in a "real world" research laboratory. However, if you plan on hosting minors (anyone under the age of 18) this summer, there are special Duke policies to protect the institution and the young scientist!

Research laboratories can have a number of physical, chemical, radiological and biological hazards which may be unfamiliar to minors, and these policies help keep young scientists safe while learning critical skills for their science future.

The Occupational and Environmental Safety Office (OESO) [safety policy](http://www.safety.duke.edu/SafetyManuals/University/1_6MinorsNon-Employees.pdf) that covers minors and non-employees in the work area (http://www.safety.duke.edu/SafetyManuals/University/1_6MinorsNon-Employees.pdf):

- No one under the age of 14 can work or volunteer at Duke Medicine.
- Children under 14 must have written OESO approval to enter a lab.
- Youth between 14-17 may not perform any work that is determined to be hazardous or potentially harmful, including:
 - work that may expose them to infectious diseases transmitted via aerosols; or
 - tasks that may expose them to blood or body fluids (infectious diseases or hazardous chemicals as listed on OESO's [Particularly Hazardous Substance List](#) available at the web link (http://www.safety.duke.edu/LabSafety/Docs/PHS_by_CAS.pdf); or
 - areas where there is potential exposure to radiation in excess of 0.1 rem (0.001 sievert) total effective dose equivalent or in excess of 10% of the limits for general employees. No minor is allowed to handle radioactive materials directly. If an AU (Authorized User) is planning on hosting minors in the lab this summer, notify the Radiation Safety Officer (684-2194) prior to their arrival; or
 - areas that are under construction; or
 - areas where ABSL2 studies are being conducted.

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CHANGES IN THE DUKE POLICY FOR MOUSE HOUSING DENSITY

The Duke IACUC has revisited the policy on mouse housing density. The review was necessary to more clearly define the Committee's expectations, while facilitating Duke researcher desires to 'do the right thing,' assuring the progress of research, meeting the federal regulatory requirements, and balancing all against our campus's disease management program! The IACUC recognized that our old policy sometimes complicated the desire, even made it difficult to 'do the right thing,' so, a re-assessment was in order.

Important aspects of the policy are defined below **(changes are highlighted)**:

- Protocols having a **high frequency of incidents** are reported to the IACUC. High Frequency is defined as the 6th or subsequent incidents within a 3 month period (from the first incident).
- An **incident** is defined when the following two (2) conditions are met:
 - A cage exhibiting excessive density has not been corrected by the PI within **two business days of DLAR notification**. Excessive density events resolved by the PI within two business days of notification by DLAR are not be counted as an incident; and
 - Any number of cages that occur within a 3 business day period of the first identified incident are **considered a single incident**. Subsequent unresolved overcrowding events are considered separate incidents if they occur >3 business days from the first identified and unresolved overcrowding incident.

See Cage Density on Page 5

Upcoming Dates & Deadlines

May 11	Amendment deadline
May 21	Amendment meeting
May 25	Amendment deadline
May 28	New Protocol meeting
June 1	New Protocol deadline (June Mtg)
June 4	Amendment meeting
June 8	Amendment deadline
June 18	BBS: AAALAC Is Coming!
June 25	New Protocol meeting

Deadlines are 5 PM on the date listed!

HANDLING & TRANSPORT OF AGRICULTURAL ANIMALS USED IN RESEARCH

By Temple Grandin, PhD
Department of Animal Sciences
Colorado State University
Part 2

Ed Note: Part 1 can be found in the April Issue of 'Animal Tracks.' Dr. Grandin is world-renowned for her abilities to sense the animal condition and has been a strong advocate for welfare approaches based upon the perceptions and needs of the animal. Her latest book 'Animals Make Us Human': Creating the Best Life for Animals' focuses on the emotional rather than the physical life of animals. To order, go to Harcourt Press. The cost is \$26.00.

Flight Zone: An important principle of livestock handling is the flight zone. The flight zone is the animal's personal space. Understanding of the flight zone can reduce stress on livestock and help prevent injuries. Animals that are trained to lead with a halter or they allow people to touch them are completely tame and have no flight zone. Animals that are not completely tame will have a flight zone and will move away when people approach.

The size of an animal's flight zone will vary depending- on its tameness or wildness. The flight zone of extensively raised cows may be as much as 50 m (164 ft), whereas the flight zone of feedlot cattle may be only 2 to 8 m. Animals housed in a research facility for several weeks will have a flight zone up to 2 m. When cattle, pigs, or sheep first arrive at a research facility, they may have large flight zones. The size of the flight zone will slowly diminish during long-term experiments if animals are handled gently. If animals are handled roughly, the size of the flight zone may increase. Animals with a large flight zone become stressed when the flight zone is deeply penetrated and they are unable to move away.

The edge of the flight zone can be determined by slowly walking up to an animal. The circle in Figure 1 represents the edge of the flight zone. When a person enters the animal's flight zone, it will turn and move away. When cattle or sheep are facing towards a handler, the handler is outside the flight zone. When the flight zone of a group of bulls was invaded in one experiment by a mechanical trolley, the bulls moved away and maintained a constant distance between themselves and the trolley (Kilgour 1971). The best place for a handler to work is on the edge of the flight zone (Grandin 1980b). This will cause the animals to move away in an orderly manner. They will stop moving when the handler retreats from the flight zone. To make an animal move forward, a person should stand in the area marked A and B on Figure 1. To make the animal back up, a person should stand in front of the point of balance (Kilgour and Dalton 1984).

IT IS TIME FOR AAALAC TO VISIT DUKE AGAIN

Once every three years, we are visited by the Site Team from the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Our next scheduled visit will occur during the fall of 2009. As we all prepare for the next visitation, you might present these questions at your next laboratory meeting. All members of your research team should be able to answer 'YES' to each of these questions. If not, please provide guidance on the specific item, or call the OAWA (668.6720) and Bill Wade will be pleased to visit with your laboratory staff and review AAALAC expectations.

1. Do we have copies of all of our approved protocols (and amendments) where our research staff can review them?
2. Are all of the research staff familiar with the protocol procedures they are working on?
3. Are we using only analgesics / anesthetics that are in our approved protocol?
4. Are our controlled substance licenses current and our controlled substance logs up to date?
5. Are we following the HUMANE ENDPOINTS listed in our protocol?
6. Are all of the surfaces in our animal use areas sanitizable?
7. Do all of our staff understand (and can they explain) Duke's veterinary care reporting system? Does our research staff know how to contact a Duke veterinarian after hours if needed?
8. Are our animals observed at least daily, including weekends and holidays?
9. Have our research staff completed the required animal training? Safety training?
10. Are all of our cages of animals labeled with CURRENT PROTOCOL cage cards and have the required PI information?

The animal program would like to help your lab be a shining star during the 2009 AAALAC accreditation site visit!

What is AAALAC?

AAALAC International is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs. AAALAC stands for the "Association for Assessment and Accreditation of Laboratory Animal Care." More than 770 companies, universities, hospitals, government agencies and other research institutions in 29 countries have earned AAALAC accreditation, demonstrating their commitment to responsible animal care and use. Duke volunteers to participate in AAALAC's program of independent assessment of our care & use activities.

For more information, visit the AAALAC website at <http://www.aaalac.org>

EFFECTS OF STORAGE TEMPERATURE AND TIME ON CLINICAL PARAMETERS FROM RAT SERUM

Carolyn Cray, Marilyn Rodriguez, Julia Zaias, et al.

Citation: Journal of the American Association for Laboratory Animal Science (Vol 48, No 2; March 2009; Pages 202–204)

Serum is often frozen and banked for analysis at a later date. This study assessed the stability of 17 analytes in rat serum during refrigeration at 4 °C and extended storage at –20 °C (frost-free and non frost-free freezers) and –70 °C. Samples were analyzed by using an automated dry-slide chemistry analyzer at time 0 and then stored as aliquots for analysis at time points including day 7, 30, 90, and 360. After 7 days of refrigeration, only creatine kinase activity had varied by more than 10% of the starting value. Freezing at –70 °C was clearly superior to –20 °C where changes were observed as early as day 30 (alanine aminotransferase changes occurs as early as day 90). Samples stored in frost-free and non frost-free –20 °C freezers did not differ significantly through day 90. Factors such as storage time and temperature should be considered when designing any retrospective study.

THE GUIDE FOR THE CARE & USE OF LABORATORY ANIMALS

Institutional Policies and Responsibilities: Monitoring the Care and Use of Animals: Food or Fluid Restriction (pg.12): When experimental situations require food or fluid restriction, at least minimal quantities of food and fluid should be available to provide for development of young animals and to maintain long-term well being of all animals. Restriction for research purposes should be scientifically justified, and a program should be established to monitor physiologic and behavioral indexes, including criteria (such as weight loss or state of hydration) for temporary or permanent removal of an animal from the experimental protocol. Restriction is typically measured as a percentage of the ad libitum or normal daily intake or as a percentage change in an animal's body weight.

Precautions that should be used in cases of fluid restriction to avoid acute or chronic dehydration include daily recording of fluid intake and recording of body weight at least once a week or more often (rodents). Special attention should be given to ensuring that animals consume a suitably balanced diet because food consumption might decrease with fluid restriction. **The least restriction that will achieve the scientific objective should be used.** In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended.

The Duke IACUC considers food or fluid restriction on a case-by-case basis, focusing on clear and demonstrable measurements for such practices.

ABSL ANIMAL HOLDING IN DUKE LABORATORIES

During the last AAALAC accreditation review (2006), the Site Team expressed concern with holding ABSL 2 animals in research laboratories across campus. Subsequent to that accreditation process, the institution established a procedure to review and approve holding of ABSL 2 animals outside of core animal care facilities. This issue, holding ABSL2 animals in PI laboratories, will be a central assessment point in the next review by AAALAC which will occur this fall!

If you have need to work with ABSL2 animals, and have not received IACUC approval for holding those animals in your laboratory, please discontinue the practice until IACUC approval has been granted. The process for gaining IACUC approval is as follows:

Step 1: Submit a 'SECTION T' protocol appendix (available on the animal program web site at http://vetmed.duhs.duke.edu/index_of_new_protocol.htm). The IACUC will review the scientific justification for housing ABSL2 animals in the laboratory. Once scientific need is established, the process moves to STEP 2.

Step 2: Assessments will be conducted by OESO, Engineering and Operations, IACUC and DLAR (this assessment is conducted as a single visit by individuals from each service) to confirm the minimums required for animal housing. This sub-committee will then approval or require modifications to secure approval of the housing space.

The standards which are used to access appropriate housing are:

- Animals are maintained in the lab area for greater than 11 hours and 59 minutes.
- Daily documentation of animal condition/care
- Adequate air flow/temp/humidity/light/heat/cooling
- Clear assigned responsibility for animal care
- SOPs for care
- Disaster plans & contact points (just in case)

You can review the requirements in greater detail in [The Guide for the Care and Use of Laboratory Animals](#). Table 2.4 provides appropriate temperatures for the housing of given species. A struggle for many laboratories is a requirement to maintain the desired temperature within a 4 degree range (i.e., for rodents 68-72 °F would be ideal) and ideal humidity should be maintained between 40-70%.

If you have any questions on measures or actions you should or could employ for your research endeavors, please contact the Office of Animal Welfare Assurance for assistance.

AMP LAUNCHES 2009/2010 PUBLIC OUTREACH FELLOWSHIP Program Honors Michael D. Hayre, DVM, DACLAM

Americans for Medical Progress (AMP) is presently accepting nominations for the Michael D. Hayre Fellowship in Public Outreach, designed to inspire and motivate the next generation of research advocates.

The Fellowship is named in honor of AMP's late former Chairman, Dr. Mike Hayre, who exhibited strong visionary leadership within the laboratory animal medicine community. Young adults aged 18-30, whether affiliated with a college or not, are invited to apply, or to be nominated. Fellowships include a stipend of \$5,000 and some for educational outreach.

Please consider whether you know someone who might be interested, or who has an idea for public outreach that would fit our criteria.

Last year Tom Holder, a leader the UK student-led campaign group Pro-Test, served as the first Hayre Fellow. He travelled throughout the United States, speaking to fellow students and others about the importance of animal research in medicine and the critical need for personal outreach on the issue. Tom also fostered - and remains very active today - in the *Speaking of Research* advocacy network. AMP hopes to find at least two new Fellows who in the academic year 2009-10 will continue to build on the tremendous outreach initiatives that Tom began last year.

For more information, contact AMP Vice President Kristen Bocanegra at kristen@amprogress.org; or visit the AMP website at www.amprogress.org.



From Page 1 ... Young Scientists

In addition, OESO requires supervisors/Pis to fill out the "Workplace Safety Statement for Minors and Non-Employees at Duke" for any minors they wish to bring into the lab. The form can be found on the [OESO Laboratory Environment page](http://www.safety.duke.edu/OESO/LaboratoryEnvironment), under the "Lab Safety Audits and On-site Evaluations" heading (<http://www.safety.duke.edu/LabSafety/Default.htm>). Once completed, this form should be sent to OESO Laboratory Safety for approval (fax: 681-7509).

Because of additional hazards that exist in animal facilities, as well as for protection of the animals, the Division of Laboratory Animal Resources (DLAR) policy is that no one under the age of 18 is permitted to enter any areas where lab animals are present. The only exceptions to this are minors who are Duke employees (i.e., summer interns) that have been given a risk assessment by EOHW medical personnel. Contact with lab animals means potential exposure to animal hair, dander and urine proteins, which can contribute to allergies. There is also the potential for exposure to infectious diseases that can be transmitted to humans or other animals. If you are considering employing minors in animal facilities this summer, please contact the main DLAR number (684-2797) for more information.

The OESO policy states that "employees who escort or supervise the activities of minors and other non-employees shall assess the potential risk of exposure to hazards and direct the non-employee's access accordingly". Assessing this potential risk includes supervisors orienting the minors to their work area, including providing any orientation training needed. For example, DLAR requires any minors working in its facilities to complete the "Hazard Awareness for Animal Facilities" training.

Summer jobs and internships can be a great way for teens to gain some valuable work experience, and it introduces them to a variety of work environments. But minors in labs require additional protections, and we need to help keep them safe while hosted in our labs. For more information on the OESO policy, call 684-2794. For more information on the DLAR policy, call 684-2797

MSDS SEARCH ENGINE AVAILABLE

OESO has a material safety data sheet (MSDS) search engine available at <http://www.safety.duke.edu/OHS/MSDS.htm>. This search engine is operated by the Canadian Centre for Occupational Health and Safety (CCOHS), and is the MSDS search engine of choice for many of Duke's peer institutions. CCOHS's database contains over 310,000 MSDS's, from more than 2,000 chemical manufacturers and suppliers. The database is updated quarterly.

In addition to the MSDS search engine, CCOHS provides a number of other valuable tools, also available at the OESO website. One of these tools is the Registry of Toxic Effects of Chemical Substances (RTECS), which provides critical toxicological information on a variety of chemicals, including drugs, pesticides, all sorts of laboratory compounds, and many more. For more information on RTECS and other CCOHS tools, please visit: <http://www.safety.duke.edu/OHS/Documents/>

- **Incidents** are tracked on a protocol basis and are not facility specific. Trending analysis may identify investigators with multiple protocols that have frequent overcrowdings (but less than 5 incidences/protocol within a 3-month period) that would also warrant meeting with DLAR, and potentially notification of the IACUC / OAWA.

The best plan for preventing a letter from the IACUC is knowing the guidelines for maximum density, and following them:

- **Mice from the Age of Weaning to Adulthood:** Mice of weaning age and above should be housed at a density of 15 square inches per mouse. For the standard Duke housing cage, this equals 5 mice per cage. (Ex: 75 square inch cage /15 square inches per mouse).
- **Heavy Mice:** If any mouse (breeder or experimental) weighs 45 grams or greater, then that cage is restricted to four (4) mice per cage.
- **Breeding mice:**
 - No more than three adults in a cage when a litter is born.
 - No more than two adults and one litter in the cage when any pups in the cage are older than ten days of age. More than one litter and two adults per cage when pups are older than ten days requires IACUC pre-approval. Any exemption must be marked on the designated cage card.
 - Pups must be weaned by 21 days of age unless an exemption has been approved by the IACUC or the DLAR veterinary staff for health concerns. Breeding cages containing pups 22 days of age or older, and without an IACUC or DLAR exception, are considered overcrowded. Any exemption must be marked on the designated cage card.
 - No more than one litter may be present in the cage. An exception to this policy is appropriate in certain 'failure to thrive' or 'poor breeder' situations:
 - More than one litter can be present in the cage regardless of the mother to which they belong if there is an IACUC or DLAR exemption in place.
 - Any exemption must be marked on the designated cage card and must include:
 - The date of birth of all litters; and
 - The IACUC or DLAR Veterinarian approved weaning date, if different than 21 days, on a designated cage card.

When litters need to be separated the mothers and litters must be observed sufficiently to determine the appropriate mother for each litter before mice are moved from one cage to another.

(Continued Next Column, This Page)

Maintaining the male in the harem post-breeding increases the risk of having overcrowded cages. Removing the male from harems after breeding has certain advantages, which include:

- Allowing the use of the male in multiple harems (decreasing the total number of animals and sharing the genetics to a larger population of offspring);
- Decrease the risk of cannibalism after birth of the offspring;
- Increase the number of pups that can be maintained in the same cage without exceeding density guidelines;
- Prevent post-partum breeding of the females (which often results in multiple litters from the same female at the same time).

Single housing of females from one week prior to gestation until gestation DOES NOT require separate IACUC approval. Refer to the Duke Animal Enrichment Policy for more information: http://vetmed.duhs.duke.edu/documents/iacuc/pdf/policy_on_enrichment_for_species_other_than_NHP.pdf

In cases of group housed rodents, where animal(s) exceed 25 grams, the veterinary staff will make a professional judgment based upon The Guide recommendations of evaluating group housed animals to achieve desirable 'individual needs, behavior, compatibility of the animals, numbers of animals, and the goals of the housing situation' (professional veterinary judgment).

The IACUC instructs DLAR to track the incidences of overcrowding, and report high frequency observations to the OAWA for investigation and IACUC consideration.

The IACUC instructs the PI to meet with DLAR representatives when 5 or more instances of overcrowding have occurred to discuss reasons for continuing overcrowding and methodologies which may be employed to lessen the frequency of overcrowded cages.

The IACUC instructs DLAR to provide a report of the PI-DLAR meeting as a means of documenting oversight corrective actions.

