REPORTING UNANTICIPATED ADVERSE EVENTS

According to the 9th Code of Federal Regulations and the NIH's Grants Policy, the institution’s animal care and use committee (the IACUC) is responsible for the care and use of all animals belonging to, our used within, the institution’s training, research, testing, or exhibition programs. To fulfill the institutional obligation (and federal expectation), any unusual animal deaths or significant health related problem caused by the environment or human error which occurs during the care or use of animals at Duke must be reported immediately to one of the following:

- Chair of the Institutional Animal Care and Use Committee (IACUC) (668.2570),
- Attending Veterinarian (684.4204),
- Office of Animal Welfare Assurance (684.4744),
- Animal Welfare Hotline (684.3535).

A trained/experienced person should take immediate action to prevent any further animal suffering. This would include removing the animals from the cause of the distress, and may include euthanizing obviously distressed animals.

If the incident occurs during non-office hours, or the above contacts cannot be immediately reached, call a Duke veterinarian. Be prepared to provide:

- A description of the incident
- Date and time of the incident
- Location of the animals (room number)
- Animal species involved
- Numbers of animals involved
- Identification of animals
- Personnel involved
- Any other relevant details.

Prevention of animal pain or suffering is an obligation for ALL of us! Persons who do observe animal pain or suffering and do not report it could potentially be held responsible by the IACUC.

Remember, failure to report animal pain or suffering is wrong—always to the right thing and protect/report animals in distress.

PROVISION OF FEED AND WATER

The Duke University animal program expects that all animals will receive the necessary quantities of feed and water to sustain life, promote health, and assure well-being, except as scientifically exempted by the IACUC for specific and defined purposes and for specific and defined periods. There are circumstances when provisions of feed/water must be reduced. The animal program has policy which described the necessary protections for animals in these situations. The Duke policy is based upon the following references:

- United States Department of Agriculture, 9 CFR Parts 1, 2, and 3.
- PHS Policy on the Care and Use of Laboratory Animals, OPRR, 1996.
- OLAW Web Site: http://grants.nih.gov/grants/olaw/
- AAALAC Accreditation Guidelines: http://www.aaalac.org
- 8th Edition: The Guide for the Care & Use of Laboratory Animals
- Lab Animal (38)10: Oct 2009. Regulatory Issues Regarding the Use of Food and Water Restriction in Laboratory Animals
- Duke Division of Laboratory Animal Resources Standard Operating Procedures necessary.

While the Duke Principal Investigator is ultimately responsible for all approved activities involving animals, the Duke IACUC expects all participants of the research protocol to follow this policy.

All participants must assure feed/water is provided according to the Duke DLAR SOP for feed/water or an alternate plan for feed/water as approved by the Duke IACUC.

CONTINUED ON PAGE 2 ... SEE ‘FEED & WATER’
The Duke IACUC defines the following terms and conditions for provisions of feed/water:

- **The Duke Standard**: The Duke Division of Laboratory Animal Resources (DLAR) establishes standard care parameters for all animals used in Duke sponsored research, testing, and teaching. For example: Required provision for standard rabbit water and feeding is listed in DLAR-HUS-2.02; required provisions for guinea pigs are found in DLAR-HUS-3.02; etc. The Duke policy for feed/water standards begins with the premise that DLAR determines 'standards of care' for providing feed/water.

- **Ad libitum or free access**: A situation where animals have unfettered access to feed or water. Ad libitum feeding is occasionally harmful to the well-being of animals (e.g., rabbits). In these cases, limit feeding shall be used to provide sufficient quantities and quality for normal growth and maintenance; water maybe provided ad libitum.

- **Reduced Water / Feed**: Defined as any provision of water or feed which is less frequent or in less quantity than listed in the Duke standards (DLAR SOPs). Restriction and deprivation are two forms of reducing water / feed. Animals placed on a reduced water / feed schedule carry the following stipulations:
  - **Minimum Frequency of Provision (Water)**: Considering the regulatory expectations for minimum frequencies of feed or water provision, the Duke IACUC expects that water is available continually or no less than twice a day for at least 1 hour each time. Any other schedule requires IACUC approval as part of the protocol. Small or younger animals generally require more frequent access to water than do older or larger animals.
  - **Minimum Frequency of Provision (Feed)**:
    - **Mice, rats**: A sufficient quantity of a nutritional ration is offered to these species at least once every 12 hours for a minimum duration of 1 hour.
    - **All other species**: A sufficient quantity of a nutritional ration is offered to these species at least once every 24 hours for a minimum duration of 1 hour or until all food is consumed.
  - **Minimum Quantity of Feed/Water**: The minimum quantity is defined by DLAR SOP according to the specific needs of the species.
  - **Monitoring While on Reduced Feed/Water**: One cannot always predict which, if any, animals on a reduced schedule of feed/water will develop problems. At a minimum, animals on a reduced intake (either quantity or frequency) of water or feed shall be monitored for:
    - Body Weight (at least every other day for juvenile animals, every third day for smaller sized adult animals and weekly for larger sized adult animals). More frequent recording may be required by the IACUC or veterinary staff.
    - Body Condition (based upon a condition score for the species)
    - Hydration Status
    - Behavioral Changes
  
  NOTE: Monitoring plasma osmolality, electrolyte levels, and/or urine specific gravity may also be valuable measures of compromised physiology.

- **Precautions When Reducing Feed/Water**: When restricting intake or volume of food or water, certain precautions are required, including:
  - Daily recording of food / water intake. Sufficient intake of nutritional compounds must be assured (restricted water / feed). IACUC approval as a Category 'E' activity is required for water / feed intake at a deprived level.
  - In the case of conditioned-response experiments, use of a highly preferred food or fluid as a positive reinforcement is recommended.
  - Animals which hibernate / estivate may have reduced supply of water / feed based upon normal species behaviors and needs. These animals also require a monitoring plan which must be described in the approved protocol.

- **Restricted Feed/Water**: A situation where access to water / feed is controlled and measured and is less than the 'standards' described in the DLAR SOPs for species. Restricted water/food does not cause significant negative impact upon animal well-being (defined as measures of physiology, activity, and general body condition). Restricted intake does not mean 'no' intake, but rather provision of a lesser volume than the animal would consume during ad lib conditions.

CONTINUED ON NEXT PAGE ... SEE ‘FEED & WATER’
Restriction is not necessarily a USDA category ‘E’ activity. There are three general methods of restricting water / feed; only one method requires IACUC approval:

1. **Restriction as a management activity:** Feed restriction is a necessary husbandry process to prevent over-eating which may result in dysbiosis and/or obesity (e.g. rabbits), either of which is potentially fatal to the unrestricted animal. Metering of food for purposes of animal well-being does not require IACUC approval. DLAR veterinary clinical opinion is sufficient.

2. **Restriction as a clinical activity:** Feed restriction is a necessary clinical care activity to prevent aspiration or asphyxiation (e.g., restricted water / feed intake is necessary to facilitate safe anesthesia induction and safe recovery from anesthesia). Restriction may also be employed for several other clinical veterinary reasons. Restricted feed / fluid intake based upon veterinary clinical requirements does not require IACUC approval. DLAR veterinary clinical opinion is sufficient.

3. **Restriction as a research activity:** Feed restriction is a necessary component to certain types of research (e.g. restriction maybe necessary to motivate the animals toward a desired behavioral goal; restriction may be used as a tool for assessing the effectiveness of research outcomes). IACUC approval is required. DLAR veterinary opinion is not sufficient for authorizing this style of restriction. The justification for a restricted water / feed plan is not required to be as rigorous as the justification for deprived water / feed schedules.

To obtain approval for restricted feed/water provisions, the IACUC requires:

A. The scientific justification for restriction of water / feed;
B. A program to monitor physiologic or behavioral parameters;
C. Criteria for removal of the animal from the study if specific endpoints have been reached (e.g., weight loss, hydration state, behavior change, etc.);
D. Urine specific gravity measurements on a frequently sufficient basis to track increasing levels of dehydration (including endpoints for removal from study), if appropriate;
   - Hematocrit measurements on a basis sufficient to track increasing levels of dehydration (including endpoints for removal from study), if appropriate; and
   - A recovery plan for re-hydration (based upon a clinically appropriate rehydration plan).

**Deprivation of Water / Feed:** Deprived intake generally means no intake of water/feed for extended periods of time which has the potential for a demonstrable impact upon animal well-being. Deprivation of water / feed for research or clinical purposes may be necessary in certain circumstances, but requires IACUC approval. When experimental situations require water / feed deprivation, consideration must be given for minimum quantities of food and fluid required to sustain reasonably normal physiology within the restricted time period. Deprivation is defined as follows:

- **Water Deprivation:** Defined as no intake for more than:
  - 8 hours: Lagomorphs
  - 18 hours: Primate (old world) species
  - 23 hours: Other species
  - 28 hours: Ruminants

  Note: Provision of 1 hour ad lib water every 12 hours is not a deprived care plan.

- **Food Deprivation:** Defined as no intake for more than:
  - 36 hours: Simple stomach animals
  - 72 hours: Ruminants

See ‘FEED & WATER’ ... NEXT PAGE
**IACUC Approval of Deprivation:** Deprivation of water / feed is a Category ‘E’ activity. The ‘threshold’ for IACUC approval of deprivation is high, and requires:

A) An assessment of ethical cost/scientific benefit
B) Clear scientific justification requiring a deprived animal for the research outcomes;
C) PI agreement to notify the DLAR veterinary staff prior to initiating the period of deprivation;
D) A program to monitor physiologic or behavioral parameters. Specifically:
   (i) Urine specific gravity measurements on a frequently sufficient basis to track increasing levels of dehydration;
   (ii) Hematocrit measurements on a basis sufficient to track increasing levels of dehydration; and
E) Criteria for removal of the animal from the study if specific endpoints have been reached (e.g., weight loss, hydration state, urine specific gravity, hematocrit, behavior change, etc.)
F) A recovery plan for re-hydration (based upon the humane endpoints).

**NOTES ON RESTRICTION / DEPRIVATION:**

1) Certain hibernating/estivating species do not consume water/feed for periods which would qualify as deprivation, but is in fact ‘normal’ for the species while hibernating / estivating. The IACUC defines hibernating/estivating animals as ‘NORMAL.’ Thus, these instances are neither deprived nor restricted. Hibernating / estivating protocols will be reviewed by the IACUC on a case-by-case basis.

2) For some species, especially rodents, restriction of water will concurrently cause the animal to eat less (and vice versa).

**NEWS FROM USDA**

The Animal and Plant Health Inspection Service (APHIS) - Animal Care Program has expanded the Animal Care Information Search (ACIS) engine. This searchable information system will give the public access to Duke inspection information (both annual reports submitted by Duke and unannounced inspection reports).

The ACIS Search Engine allows users to search using multiple criteria (e.g. two or more states, two or more CFR section violations, two or more licensee types). All data generated by a search can be electronically sorted and exported for analysis.

Data from Form 7023 (the Annual Report), submitted by all research facilities are available for electronic searching, sorting, and exporting for analysis. Column E explanations and exceptions for FY 2010 and future years are also available through the ACIS Search Engine.

The [ACIS Search Engine](#) can be found on the USDA website or by clicking here.

**FROM THE LITERATURE**

Cage Change Influences Serum Corticosterone and Anxiety-Like Behaviors in the Mouse
Skye Rasmussen, Melinda M Miller, Sarah B Filipski, and Ravi J Tolwani

Environmental variables and husbandry practices can influence physiology and alter behavior in mice. This study evaluated the effects of cage change on serum corticosterone levels and anxiety-like behaviors in C57BL/6 male mice. The authors looked at the effects of different handling methods (forceps transfer, gentle transfer with gloved hands, and a passive transfer technique) that did not involve active handling. They found significant increase of serum corticosterone 15 min after cage change; however, at 60 min after cage change, levels were comparable to those of unmanipulated mice. Although the effects were transient, cage change altered anxiety-like behaviors in the open field when behavioral testing was performed on the same day. These results demonstrate that the timing of cage change can influence behavioral results, an effect that is an important consideration for rodent behavioral studies. To read more about these findings, see the JAALAS; Vol 50: Num 4. July 2011
NEWS FROM OLAW

PODCAST: WRITING YOUR VERTEBRATE ANIMAL SECTION

A new All About Grants Podcast produced by the NIH Office of Extramural Research (OER) featured Dr. Pat Brown, Director OLAW, who explains what must be included in your grant application if you use vertebrate animals in your research. Listen to Writing Your Vertebrate Animal Section (MP3) or read the transcript (HTML).

A recording of the OLAW Staff Online Seminar, Writing a Good Assurance held June 9, 2011 has been posted on the OLAW website. The seminar recording and supporting materials can be found on the Education Resources page.

OLAW has updated the Training Materials webpage. New additions to the page include a Spanish version of the “Working Safely with Nonhuman Primates” video plus the “Mouse and Rat Humane Restraint and Experimental Techniques” CD developed by the NIH Office of Animal Care and Use. Information about how to download or order these new training materials can be found on the OLAW homepage under resources, or go to: http://grants.nih.gov/grants/olaw/TrainingVideos.htm

FROM THE LITERATURE

Ethics and Animal Numbers: Informal Analyses, Uncertain Sample Sizes, Inefficient Replications, and Type I Errors Douglas A Fitts

To obtain approval for the use vertebrate animals in research, an investigator must assure the IACUC that the proposed number of animals is the minimum necessary to achieve a scientific goal. Frequently, researchers use a power analysis, which is considered the most accurate method when the outcome is known before the study, which it rarely is. A ‘pilot study’ is appropriate only when the number of animals used is a tiny fraction of the numbers that will be invested in the main study because the data for the pilot animals cannot legitimately be used again in the main study without increasing the rate of type I errors (false discovery). Traditional significance testing requires the investigator to determine the final sample size before any data is collected and then to delay analysis of any of the data until all of the data are final. An investigator often learns at that point either that the sample size was larger than necessary or too small to achieve significance. Subjects cannot be added at this point in the study without increasing type I errors. In addition, journal reviewers may require more replications in quantitative studies than are truly necessary. Sequential stopping rules used with traditional significance tests allow incremental accumulation of data on a biomedical research problem so that significance, replicability, and use of a minimal number of animals can be assured without increasing type I errors. To read more about these findings, see the JAALAS; Vol 50: Num 4. July 2011

Disposing of Expired Controlled Substances

The DLAR pharmacy now operates under a North Carolina and a United Started DEA distributors license. This means that controlled substances purchased from DLAR by Duke Principal Investigators must cannot be returned to DLAR for disposal. The DLAR Pharmacy DOES NOT have a ‘reverse distributor license.’

What are the options for disposal?

A. Inject the expired controlled substance into a deceased (not alive) research animal. Dispose of the carcass in the standard manner.
B. Mix the expired controlled substance with ‘kitty litter’ and toss the ‘wet litter’ in the trash can.
C. Toss the expired controlled substance in the incinerator.

NOTES:

1. Options A, B, or C require an observer sign the log sheet documenting the disposal.
2. Keep the log sheet for 3 years past the disposal date, after which time, you may destroy the document.
3. DO NOT dispose of controlled substances by pouring into the lab sink drain!
**APPROVED SECONDARY METHODS OF EUTHANASIA**

The Duke University IACUC policy on Euthanasia outlines the approved secondary methods of euthanasia following CO2 asphyxiation. Appropriate secondary methods include:

- Bilateral Thoracotomy
- Collection of tissues sufficient to assure that the animal will not recover
- Exsanguination

**CERVICAL DISLOCATION IS NOT AN APPROVED METHOD OF SECONDARY EUTHANASIA**

It is particularly important to note this when developing protocol amendments. An approved alternative must be listed or a request for exception to approved methods, with scientific justification, when submitting documents to the IACUC for approval.

Researchers should ensure all staff are notified of this policy and aware of the appropriate techniques they must employ.

If you have any questions about the use of CO2 or secondary methods following CO2 exposure please contact the Office of Animal Welfare Assurance.

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**CONCORDANCE REVIEWS**

A concordance review is a comparison between the grant submitted to the funding agency and the protocol reviewed by the IACCUC. Concordance reviews are required by many (but not all) granting agencies—especially federal ones. Generally, the granting agencies require an signed concordance letter PRIOR to release of the funds for the grant, so it is important to plan ahead!

Concordance reviews are performed by the OAWA/IACUC and require 5-7 business days to complete. Why so long? Because a concordance review compares each and every procedure stated in the grant with the procedures approved in the protocol, and they must match! To be concordant, everything you told your granting agency you would do with animals, even if it will not be done until the 4th or 5th year, must be IACUC approved before the concordance letter can be generated.

The OAWA/IACUC requires the whole grant EXCEPT the bio sketches and the budget pages. It is preferred that an electronic copy be provided when the protocol is submitted (if you have the grant at that time). If not, a concordance review can be done at any time, but it will take the 5-7 business days to complete.

The secret? PLAN AHEAD! If you think you MAY need a concordance review, request it up front, because these reviews cannot be rushed, and do require time to complete.

www.4allfree.com

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**Dysfunctional Germ Family**

YOU MAKE ME SICK?

GASPIRITZ
Americans for Medical Progress, a national non-profit that stands in support of biomedical research, invites applications for the Michael D. Hayre Fellowship in Public Outreach for the 2012-2013 academic year.

Named in memory of Michael D. Hayre, DVM, DACLAM, the Hayre Fellowship supports innovative projects in peer education about the necessity for animal research.

Applications open: January 15, 2012

For full details about the Hayre Fellowship visit: www.amprogress.org/HayreFellowship.