AVMA GUIDELINES FOR THE EUTHANASIA OF ANIMALS
(2013 EDITION)

The long-awaited update of the AVMA Euthanasia Guidelines has arrived! On March 1st, the AVMA released an update to a seminal document for animals care programs. While the Duke Animal Program continues its detailed review of the new document, a quick broad brush assessment indicates that the impact upon Duke researchers will be minimal, with the greatest impact coming to those researchers using carbon dioxide as the primary method of euthanasia. While note 100% clear at press time, it is certain that the prior method of using a pre-charged chamber for CO₂ euthanasia will no longer be accepted. The IACUC, OAWA, and DLAR are formulating final plans for new training and new expectations to be in full compliance with the Guidelines. We’ll be providing those updates and recommendations within the next several weeks.


Wishing you a successful research month,
REPORTING MISSING or ESCAPED ANIMALS

Even under the most controlled circumstances, adverse events may occur. When an animal escapes the holding cage, it is not acceptable to consider it ‘returned to the wild’ or ‘free to roam the building.’ Measures to recapture the animal must be initiated immediately. The goals of recapture are to:

1. Prevent an injury to an animal unaccustomed to the out-of-doors;
2. Prevent transgenic animals from passing their modified genes to other animals; and
3. Prevent the spread of potential pathogens (if the animal is infectious).

A few things to remember when recapturing animals:

- A rodent which has escaped should not be handled by hand. Use a hard container (e.g., a cup or empty cage) to recapture animals.
- Animals found in a trap or on the floor must be placed in a clean cage with food and water.
- A label using the word “compromised” must be affixed to the cage. This denotes that the animals may not be healthy and should be handled as if infected.
- A DLAR veterinarian must be notified immediately after the animal is captured.
- If DALR can determine the PI associated with the recaptured animal, the PI will be notified.
- If you suspect an animal is missing, check the room mortality log (has the animal died and the carcass removed for refrigerated storage?).
- If you suspect an animal is missing, or you know it is missing and cannot find the carcass, contact the DLAR supervisor.

It is especially important to notify DLAR management if the missing animal is a transgenic animals, KO/KI, or an animal with recombinant DNA. According to NIH policy, loss of these animals may require notification of the NIH Office of Laboratory Animal Welfare.

SUBSTANTIVE CHANGES IN THE 2013 EDITION OF EUTHANASIA GUIDELINES

SPECIES-SPECIFIC SECTIONS: In addition to the sections describing various techniques in detail (inhaled, noninhaled and physical methods), specific advice is provided for the euthanasia of companion animals, laboratory animals, animals farmed for food and fiber, equids, avians, fish and aquatic invertebrates, and captive and free-ranging nondomestic animals. Recommendations relating to a particular species may be located within the guidelines by using Appendix 1 “Agents and methods of euthanasia by species,” which references the section(s) within the document that should be consulted. New figures and tables provide helpful guidance in the application of specific techniques.

WIDER CONSIDERATIONS: Information has been included about key concerns outside of the immediate performance of a euthanasia technique (i.e., euthanasia is approached as a process, rather than as an isolated event). This includes advice on ethical decision making, consideration of the various environments in which euthanasia is conducted, handling of animals, confirmation of death, and disposal of animal remains. Collection of animals for scientific investigations, euthanasia of injured or diseased wildlife, and handling of animals under field conditions are also addressed.

SLAUGHTER AND DEPOPULATION IN SEPARATE DOCUMENTS: One area identified as needing additional guidance upon review of the last iteration of the Guidelines was depopulation (i.e., the rapid destruction of large numbers of animals in response to emergencies, such as the control of catastrophic infectious diseases or exigent situations caused by natural disasters). Depopulation may employ euthanasia techniques, but not all depopulation methods meet the criteria for euthanasia. The AVMA will create two additional documents to address these shortcomings:

- Guidelines for the Depopulation of Animals.
- Guidelines for the Humane Slaughter of Animals.

‘CONDITIONALLY ACCEPTABLE’ REPLACED WITH ‘ACCEPTABLE WITH CONDITIONS’: Techniques are categorized as ‘acceptable’, ‘acceptable with conditions’, or ‘unacceptable’. The replacement of ‘conditionally acceptable’ with ‘acceptable with conditions’ is intended to signal that ‘acceptable’ and ‘acceptable with conditions’ techniques are to be considered equally satisfactory so long as the stated conditions are met.

PODCAST

The Foundation for Biomedical Research’s (FBR) provides a regular podcast of new and interesting research advances at the link: http://fbresearch.wsol.net/podcasts.aspx
**Q:** How quickly can I get my amendment to an existing protocol completed?

**A:** The Duke IACUC uses four (4) parallel processes to minimize the delay between submission and a response (hopefully approval). These processes are:

- **Category I:** Significant Changes reviewed by the Full Committee Review (FCR). These proposals are sufficiently serious to require documented deliberation of the full committee. Since the IACUC only meets monthly, these reviews may take up to 28 days to accomplish. While any style of proposed change may be reviewed by this process, the more common examples subjected to FCR include:
  - Request for ABSL-2 or greater PI-Managed Housing (Housing for greater than 12 hours in a non-DLAR facility). Complete Section T of the protocol template for review with the amendment.
  - Exemptions to The Guide or other regulatory guidance or institutional policy. A completed Section U (Exemption) of the protocol template is required for this style of amendment.
  - Amendments involving primates, dogs, or cats.

- **Category II:** Significant Changes (SC) reviewed by the SC Subcommittee or by Full Committee Review (monthly). These categories of proposals are less serious and generally do not require Full Committee Review. The SC Subcommittee meets on the 1st and 3rd Thursdays each month. Examples of proposals which qualify for this Category II process include:
  - Addition of a new species.
  - Any increase in animal numbers of non-human primate, swine, canine, and feline species.
  - Increase in animal numbers > 20% of the approved animal numbers for any species other than non-human primate, swine, canine and feline species. Note: Any increase in animal numbers requires IACUC approval.
  - Change of a non-survival to survival surgery.
  - Changes in anesthetic drug regimens which increase the potential for pain or distress.
  - Changes in analgesic drug regimens which increase the potential for pain or distress.
  - Changes in humane endpoints which increase the potential for pain or distress.
  - Increased proportion of expected animal deaths.
  - Increase in the duration of pain, discomfort, or distress to an animal.
  - Hazardous agents (as defined by OESO) administered to animals.
  - Principal Investigator (PI) change.
  - Addition of neuromuscular blocking agents.
  - Request for ABSL-1 PI-managed housing (Housing for greater than 12 hours in a non-DLAR facility). Complete Section T (PI Managed Housing) of the protocol template for review with the amendment.

- **Category III:** Minor Amendments reviewed by the IACUC Leadership Review (ILR) including a ‘veterinary review’ as part of this ILR process. These categories of proposals have a potential welfare \ well-being impact, but generally the outcome is more favorable and in the best interest of the animal. These reviews generally take 5-7 business days to complete. Examples of proposals which qualify for this Category III process include:
  - Changes in anesthetic drug regimens which decrease the potential for pain or distress; or due to unavailability of protocol-approved medications.
  - Changes in analgesic drug regimens which decrease the potential for pain or distress; or due to unavailability of protocol-approved medications.
  - Changes in humane endpoints which decrease the potential for pain or distress.
  - Addition of a procedure which should decrease the proportion of adverse outcomes (e.g., post-procedure death, distress, or pain).
  - Changes in animal care and/or monitoring practices which increase the frequency or methodology of monitoring.
  - Addition of sample collection (as long as sample collection volumes do not exceed IACUC-accepted standards for the period of collection, such as the 24 hour maximum for blood collection from mice).
  - Repetition of an already approved experiment (where no additional animals are required) to re-affirm experimental data or replace questionable data.
  - Addition of noninvasive sampling/analysis (e.g., MRI, motion sensor, etc.).
  - Change or addition of euthanasia procedures from an AVMA ‘Acceptable Method to an AVMA ‘Conditional Method’ (AVMA Guidelines).
  - Requests for delayed weaning (generally rodents) as described in the Cage Space Requirements for Mice Policy or the Policy for Enrichment for Species Other Than Non-Human Primates.
  - Requests for single housing (e.g., aggressive male rodents) for animal welfare purposes as described in the Cage Space Requirements for Mice Policy or the Policy for Enrichment for Species Other Than Non-Human Primates.

- **Category IV:** Minor Amendments reviewed by the ILR. These categories of proposals have no direct animal welfare \ well-being impact). These proposals are reviewed generally take 1-2 business days to complete. Examples of proposals which qualify for this Category IV process include:
  - Transfer of animals to another protocol where animals (same stock / strain) are already approved on that study.
  - Addition or deletion of personnel to an active approved protocol.
  - Adding or changing the site of animal procedures (less than 12 hours)
  - Change in or addition of euthanasia procedures to an AVMA ‘Approved Method’ (AVMA Guidelines).
  - Increase in animal numbers < 20% of the approved animal numbers of species other than non-human primate, swine, canine, and feline species. Note: Any increase in animal numbers of primates, swine, canines, and feline species requires IACUC approval (e.g., Category I or II processes).
  - Addition of another strain / stock of the same animal species.
  - Addition of a location for PI managed housing if an approved Section T is already in place in the protocol (Step 1: Scientific Justification has already been approved). The amendment will be processed according to Step 2 of the Policy on PI Managed Housing Facilities detailing facility inspection of the new area.
A recently published report of the limitations of mouse models ("Genomic responses in mouse models poorly mimic human inflammatory diseases") has been used by the Humane Society of the United States (HSUS) to justify their drive to end all animal use in research. However, this report in PNAS was a further refinement of our knowledge concerning which animal models are useful for studying various diseases, and is not a blanket condemnation of animal research at all.

The notion that research animals are only useful when they mimic human complexity is simple-minded. Much modern research is done on other organisms because they possess a basic primitive trait and do not have a complexity that confounds experiments.

For instance, Tetrahymena thermophila is a primitive one-celled organism that allows us to study the ultrastructure, physiology, development, and biochemistry of a cell without the interference of our added-on complexity. This is based on "functional conservation"—the concept that a genetic solution to the chemical problems of living is usually solved only once in evolution, and remains operational in the lineage up to humans today.

Nobel Laureate Andre Lwoff grew Tetrahymena in pure culture in 1923; this led to the later Nobel-prize winning discovery of ribozymes, as well as other discoveries of lysosomes, telomeres, etc. It should not be surprising that the vast majority of Nobel Prizes in Physiology or Medicine have been based on animal research.

Researchers have no reason to use research animals that do not contribute to our understanding. Just as they have lists of animals that are useful for researching certain diseases, researchers have a longer list of animals that are not appropriate. The recent PNAS paper merely advances that knowledge. Indeed, the breakthroughs in the last two decades have spawns a resurgence in both basic and applied research, and expanded the frontiers for animal research.

Critical roles of animals in research include:

- Sequencing the genome of a primitive sponge reveals genes for the first signaling pathways and structures of animals, including early genes implicated in cancer.
- Hydra and comb jellies allow researchers to understand body patterning, the origin of epithelia, and regulation of development.
- Flatworms help us understand regeneration of body parts, stem cells, and the beginning of complex behavior.
- The roundworm C. elegans continues to be an excellent model for understanding genetic control of development and physiology; it was the first multicellular organism to have its genome completely sequenced, and revealed that some cells must die on cue for normal development ("programmed cell death").
- Segmented worm larvae have the simplest eyes known; some species are bioindicators of pollution.
- Without the huge neurons of the squid, we could not understand the process of nerve signal transmission because human axons are several orders of magnitude smaller.
- The 20,000 neurons of the sea hare Aplysia allow us to associate nerve cell chemistry with behavior.
- Millions of fruit flies drive our understanding of genetics.
- Transparent external sea urchin eggs help us understand fertilization.
- Lampreys are used in spinal cord research.
- African clawed frogs are critical in developmental biology because they have large embryos and a high tolerance for physical and drug manipulation.
- The chicken is used for developmental studies—an excellent model for micromanipulation in tissue grafting and research on the overexpression of gene products.
- The zebra finch is used to study birdsong and non-mammalian hearing systems.
- Ferrets made recent news in studies of mammal-to-mammal transfer of the bird flu virus.
- Downsizing of the chimpanzee research population still recognizes that there are some research applications for which these animals are the only option.
Court: University of Florida must disclose locations of animal research labs

The University of Florida has to release the locations of its animal research labs after a court battle with animal rights activist Camille Marino ended with an appeals court ruling that the locations are public record. The First District Court of Appeal filed the opinion this week, reversing a lower court’s ruling. Marino, part of the animal rights group Negotiation Is Over, had already won one legal battle over the release of the records.

UF spokesman Chris Moran said UF was disappointed in the ruling but would remain vigilant in protecting its researchers. “This is about the safety of our people,” he said. “That's what this is about.”

Last year, several UF employees said they had been harassed or threatened after their home addresses were posted on the website for Negotiation Is Over.

Marino pursued the appeal last year while awaiting trial for an unrelated case in Michigan. She was arrested in Gainesville in February 2012 during a protest outside a ceremony at Emerson Alumni Hall at UF. Gainesville Police arrested her on an out-of-state warrant from a case related to her protests of a Wayne State University researcher. Moran said UF will comply with the records request, and the university has no plans to appeal any further.

"As far we're concerned, the case is now done and closed," he said.

IACUC Semiannual Site Visits
(Spring 2013)

The remaining IACUC semiannual site visit schedule for Spring 2013 is:

- **March 14**: CARL; Eye Center; DURF; Independence Park
- **March 15**: Duke Marine Lab
- **March 21**: CCIF
- **April 4**: Foster St.; Bio Sci; French Science
- **April 11**: Duke South; CIEMAS, GHRB
- **April 18**: Vivarium; MSRB2
- **May 2**: MSRB, Lemur Center, Ecotox

Another case of "no-animal-research, no-cure" is leprosy (now called Hansen Disease). We could not develop an effective drug to the mycobacterium that causes this disease because we did not have an animal model to test potentially risky candidate drugs. When the nine-banded armadillo was discovered to harbor the disease in the 1970s, effective drugs were developed and Western leprosariums closed.

Alexander Fleming tested penicillin in a Petri dish and decided it did not work as a clinically effective antibiotic. But Florey and Chain tested it against mice and found it was effective. The history of biological research contradicts any claims that we can set aside all animal research and just use simulations.

What makes science distinct from non-science is its constant referencing to the real world. No computer simulation, tissue culture, or other model begins to approach the complexity of whole living organisms. Suppressing science research has consequences as well. Failure to have used animal models would mean nearly all vaccines, antibiotics and other pharmaceuticals could not have been developed. Are advocates of ending animal research willing to bear the responsibility for continuing polio, leprosy, etc.?

With the recent breakthrough in sequencing the genomes of organisms from protozoans to humans, whole new fields of study have opened up. We are in a new golden age of discovery connecting DNA to its physical expressions. New fields of proteomics, transcriptomics, metabolomics, and other "omics" require an expanded research effort to integrate the new molecular data with the biology of whole organisms. Our understanding of life processes forms a vast fabric, the threads of which tie together protists and sponges and worms and mice and chimpanzees and humans.

The H.S.U.S. no-animal-research position also ignores animal research that allows us to provide better care for our animals by advancing veterinary science. And ecotoxicity testing, based on the diversity and complexity of animal systems also serves animals by protecting our shared environment. To end animal use in research makes no more sense than ending plant research or research in the physical sciences. And advocacy for doing so threatens your health and the health of future generations.

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**RESEARCH SAVES**

The Foundation for Biomedical Research’s (FBR) quarterly biomedical magazine *Research Saves* showcases medical and scientific breakthroughs with foundations in animal research and strong human interest elements. All articles are submitted by universities, nonprofits and companies across the country. Each magazine also includes a full-length DVD, poster or educational program.

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**Q: Why does the IACUC require me to assess pain and distress before the experiment is performed?**

**A: The federal agencies require the IACUC review animal protocols to ensure that pain and distress are minimized in laboratory animals. The IACUC must assure that the principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animal and has provided a written narrative description of the methods and sources used to determine that alternatives were not available.**

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**REVIEW OF GRANTS & CONTRACTS SUBMITTED TO THE PHS**

From the Institutional Animal Care and Use Committee Guidebook**

In order to approve a protocol that involves the use of animals, the IACUC must review the proposed care and use of animals and determine that federal criteria have been met.

PHS requires that the project be conducted in accordance with the *PHS Policy*, the AWA, the *Guide*, the institution’s Assurance, and all other applicable federal statutes and regulations related to animals. The project should also comply with all institutional policies.

Most IACUCs (including the Duke IACUC) require use of a standardized protocol application form to assist the investigator in providing the information necessary to ensure compliance. While there is no explicit requirement for the IACUC to do a side-by-side comparison of the information contained in the IACUC protocol review form and the information submitted to PHS, it is imperative that the protocol that the IACUC approves is consistent with the information submitted to PHS. Institutions should devise a mechanism to verify that consistency.

If the IACUC requires changes to the protocol that are not reflected in the grant application, then the PHS funding component must be notified in the follow-up certification of IACUC approval. Institutions are required to provide PHS with the date of IACUC approval.

There is no provision for providing a contingent approval date; the date provided must signify full approval by the IACUC. If an institution has a PHS Assurance, then in most cases the PHS allows a 60-day grace period following the receipt deadline date during which the investigator may secure IACUC approval; otherwise, the application cannot be peer reviewed.

If the IACUC review occurs subsequent to the grant submission, then a letter verifying IACUC approval, and stating any modifications required by the IACUC, must be submitted to the funding agency. This grace period is nonexistent for some non-federally funded projects and investigators are required to submit evidence of IACUC approval coincident with the grant or contract submission.

**You can view the entire text of this publication online at**