DENOVO REVIEWS
(also called 3-Year Re-Write)

Frequently a researcher will engage in animal research activities that may continue for several years. Under NIH/PHS Policy the maximum interval between IACUC review and approval is three years. A de novo review (complete new protocol) is required at least every three years for continuation of the animal work past year three. This review must encompass all of the criteria in the NIH/PHS Policy (Section IV.C.1.a.-g.).

While many institutions have historically talked about a protocol lifespan as 36 calendar months, recent actions by the NIH/PHS have clearly defined three years as 1,095 days—not 36 calendar months. Superficially, that wouldn’t seem to be a problem, but the Gregorian calendar does not place the same week and month on the same day three years hence. Using the calendar approach, and considering that the Duke IACUC usually meets on the 4th Thursday of the month, three calendar years is somewhere between 1,095 and 1120 days. Any work beyond 1,095 days on the original protocol may not be funded by NIH grants.

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Upcoming Dates & Deadlines

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Deadlines are 5 PM on the date listed!
The long-awaited update of the AVMA Euthanasia Guidelines has arrived! On March 1st, the AVMA released an update to a seminal document for animal care programs. 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.

The NIH responded with publication of a notice indicating full compliance with the new AVMA guidelines not later than 1 September 2013. The full text notice is available on-line: Implementation of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2013 Edition has been published in the NIH Guide for Grants and Contracts, NOT-OD-13-048.

The Duke Animal Care and Use Program has been updating the current institutional policies regarding euthanasia of research animals. While 95% of our prior policies saw no change, there were subtle but significant modifications required to be in full compliance with the newly published guidance. While the NIH has granted a ‘grace period’ through September 2013, it is our goal to achieve full compliance during the summer months.

The most notable change in the policy is the removal of pre-charging the cage/chamber with CO2 prior to the placement of animals. The new CO2 euthanasia procedures will also require the use of a flow-meter to regulate the % concentration of CO2 being introduced into the cage/chamber. The maximum flow rate for CO2 euthanasia is 30% volume exchange. This is a critically important number as situations which exceed 30% flow will be (after the grace period ends) an NIH non-compliance, and probably require reporting to both the NIH Office of Laboratory Animal Welfare and the Project Manager for the affected grant.

The Duke IACUC has thus far:
1. Updated the Policy on Euthanasia.
2. Updated the Web Module Training
3. Established a three-year renewal for Web Training for all personnel listed on a protocol wherein CO2 is an approved method of euthanasia.

The training requirement for a 3-year re-do is effective immediately. The updated web module is on the OESO Training Web (same name as before—CO2 Euthanasia). You and members of your laboratory may complete the updated module at any time but must have completed the module prior to 1 July 2013! The exception is if you have a protocol up for renewal in May or June, then the new module must be completed immediately. Everyone must complete the module by July 1, regardless of when your protocol expires. Failure to complete the module (for persons listed in section A-3 of protocols having CO2 as a method of euthanasia), will result in a delayed approval process (while the IACUC waits for protocol staff to complete the training).

Within DLAR-managed facilities, custom lids are (and have been) in use for several months. Many researchers have been using the new DLAR processes for several months. Previously, it was acceptable to use either system, but effective 1 July, only the new procedures are considered acceptable.

In non-DLAR-managed facilities, a system must be in place to use the new procedures prior to 1 July 2013. The new procedures require a specialized lid and flow system. OAWA and DLAR have partnered to manufacture CO2 euthanasia cage lids for researchers to use. A member of your research team may obtain one of these lids at no cost by attending a Brown Bag Seminar on CO2 Euthanasia. You may also construct your own system, as long as it meets the requirements of the AVMA guidelines.

Flow meters are being selected as ‘most preferred.’ The preferred flowmeters, and ordering information, will be available very shortly.

The OAWA is also provided Brown Bag Seminars (BBS) as an alternative to the web module on CO2 Euthanasia. Attending a BBS will grant credit for the module. The dates for three scheduled BBS’s are:
- 8 May
- 10 May
- 22 May

The PowerPoint handouts for the new CO2 euthanasia training may be downloaded from the Duke animal program web site (Training Page).
USE OF NON-PHARMACEUTICAL GRADE SUBSTANCES IN LABORATORY ANIMALS

The use of pharmaceutical-grade substances in laboratory animals ensures that the substances administered meet established documentable standards of purity and composition. This in turn helps ensure research animal health and welfare, while supporting valid experimental results. The use of lower grade substances/compounds with undefined, higher levels of impurities, or poorly formulated non-commercial preparations can introduce unwanted experimental variables or even toxic effects, and therefore should be avoided if at all possible.

Although pharmaceutical grade substances should be used in experimental animals whenever possible, the use of non-pharmaceutical-grade substances in experimental animals is an acceptable practice under certain circumstances. For example, new investigational compounds are not pharmaceutical grade (if they were, they wouldn’t be investigational).

The NIH Office of Laboratory Animal Welfare (OLAW) and the United States Department of Agriculture (USDA) determined that the use of non-pharmaceutical-grade materials must be based on:

1. Scientific necessity,
2. Non-availability of an acceptable veterinary or human pharmaceutical-grade compound, and
3. Specific review and approval by the institutional IACUC.

Cost savings alone is not considered an adequate justification for the use of non-pharmaceutical-grade substances in laboratory animals.

OLAW has also stated that while the possible implications of the use of non-pharmaceutical grade substances in non-survival studies appears less evident, the scientific issues remain the same and professional judgment, as outlined above, must still apply.

It is important to understand that guidelines regarding non-pharmaceutical substances applies to all components of a medicament, carrier, compound, or mixture — whether active and inactive. Therefore, the vehicle used to facilitate administration of a compound is as important of a consideration as the active compound in the preparation. OLAW has also stated that when compounding products, if any component of the compounded product is non-pharmaceutical, then the entire compound is non-pharmaceutical.

DEFINITIONS: While there is a bit of variability between agency definitions, for the purpose of the Duke Animal program, the following definitions apply:

- **Pharmaceutical grade compound:** Drug, biologic, reagent, etc. which is approved by the FDA, or for which a chemical purity standard has been written/established by USP/NF or the BP.
- **USP/NF: United States Pharmacopeia/National Formulary:** A legally recognized compendium of standards for drugs, published by the United States Pharmacopeia Convention, Inc.. Revised periodically; it includes assays and tests for determination of strength, quality, and purity. USP materials are considered pharmaceutic grade, but one level below FDA-approved pharmaceutics, and equal to BP grade substances.
- **BP: British Pharmacopeia:** A legally recognized compendium of standards for drugs, published by the British Pharmacopeia. Revised annually, it includes quality standards for United Kingdom medicinal substances used in pharmaceutical research, development, manufacture and testing. BP materials are considered pharmaceutic grade, but one level below FDA-approved pharmaceutics, and equal to USP/NF grade substances.
- **Analytical grade chemical:** Any drug, biologic, reagent, etc. for which there is a Certificate of Analysis, generally reporting ~99% purity of the material. Analytical grade agents are not considered pharmaceutical grade.
- **Non-availability:** One criterion which may be used to justify non-pharmaceutical substance use, and is defined as not commercially available from an active US vendor.

HIERARCHY OF AGENT SELECTION: When selecting compounds for use in animal-facilitated activities, the following order of choice should be applied:

1. FDA-approved veterinary or human pharmaceutical substances;
2. FDA-approved veterinary or human pharmaceutical substances used to compound a needed dosage form;
3. USP/NF or BP pharmaceutical grade substance used in a needed dosage form (also includes compounded products from sources such as compounding pharmacies);
4. Analytical grade chemical used to compound a needed dosage form. Note: Analytic grade is not pharmaceutical and requires specific IACUC justification.
When reviewing a proposal to use non-pharmaceutical grade substances in animals, the IACUC must consider:
- Animal welfare;
- Impact upon scientific validity;
- Potential for contamination (e.g., metabolites, toxins);
- Safety of the proposed product;
- Efficacy; and
- Inadvertent introduction of confounding research variables;
- Grade/purity
- Formulation of the final product;
- Sterility;
- Pyrogenicity;
- Stability;
- pH;
- Osmolality;
- Site/route of administration;
- Pharmacokinetics;
- Physiological compatibility;
- Storage conditions; and
- Quality control.

The ability to use non-pharmaceutical agents when a similar agent exist in a pharmaceutic form is becoming increasing restricted by the funding and oversight groups. Researchers must assure they have explored all options regarding using pharmaceutical qualified products prior to suggesting use of a non-pharmaceutic agent. Prior use of a non-pharmaceutical substance is not an acceptable argument for continued use of the same products.

SOURCES OF PHARM-GRADE CORN OIL

One common carrier used in animal-based research is corn oil. Historically, food grade corn oil has been acceptably used, but oversight agencies have restricted such practices for products, like corn oil, which are increasingly available as a pharmaceutical grade product.

Current regulatory guidance defines corn oil from a supermarket (e.g. Kroger, Harris Teeter, Walmart, etc.) as unacceptable for animal-facilitated research. Food grade does not equal pharmaceutical grade.

For researchers requiring corn oil for animal-facilitated activities, one available source is:

⇒ VWR: VWR # 70000-136 Price = $69.28; Corn Oil NF – 500ml Amber Glass; Made by Spectrum (Product # is CO136)
The Academy of Surgical Research Offers Certification Which Meets the Needs of the Oversight Agencies

The 8th Edition of The Guide requires institutions document training and certify animal related skills. One challenging area to accomplish documentation is with surgical and surgical support skills. The Academy of Surgical Research now offers surgical-associated skills certification; which is accepted by the Duke IACUC, funding, and oversight agencies. The Academy offers three different styles of certification, including (a brief review and requirement for each style is provided):

**Surgical Research Anesthetist (SRA) Certification:**

- **Case Log:** The case logs for the SRA will consist of a minimum of 30 anesthetic cases, with no more than 15 in any one species. Acceptable cases must be longer than 30 minutes of surgical duration, have had the applicant present from induction through recovery, have the applicant primarily acting as the anesthetist and have a minimum of monitoring to include heart rate, respiratory rate, body temperature, and at least one of the following parameters: blood pressure, SaO2, or capnography. At least two different species must be included. Rodents may be included provided that the above monitoring was performed with the applicant present. Applicant may not deliver the patient to the surgeon and then leave the room. Acceptable survival surgical procedures are required for the SRA logs and narratives, which involves a minimum of 72 hours of survival post-surgery before euthanasia. An exception may be made for complex acute procedures which require the applicant to adhere to a rigorous anesthetic management protocol with multiple drugs being administered to maintain the patient (these exceptions will be made at the discretion of the certification committee on a case by case basis).
- **Narratives:** The applicant must submit two narratives which detail two of the cases from the applicant’s anesthesia log. Each narrative should comprehensively describe all aspects of the anesthetic protocol, including the following:
  - Drug Regimens including doses, routes of administration and timing related to the surgical procedure (prior to surgery, during the procedure, etc.) for pre-anesthetic, anesthetic, analgesic, and antibiotic drugs.
  - Justification for the anesthetic regimen used.
  - The methods used to monitor the animal’s condition and anesthetic depth pre-operatively, intra-operatively, and immediately post-operatively.
- Additional requirements include:
  - A description of the animal’s condition during the procedure in linear format including what adjustments were made in drug administration, the reasons for these changes, and the results.
  - Animal preparation for surgery including areas clipped, surgical positioning, antisectic prep regimen, and fluid therapy.
  - An overall assessment of the efficacy of the anesthetic protocol for the animal and what changes, if any, were made based on the results.
  - Any complications which occurred during the anesthetic period and how they were addressed.
  - Post-operative monitoring and pain assessment including species appropriate signs of pain and distress and duration of monitoring including follow-up procedures and care.

**Surgical Research Technician (SRT) Certification:**

- **Case Log:** The SRT case log will include a minimum of 12 survival, aseptically performed procedures defined as “minor” surgical procedures. At least two different procedures must be included. Non-survival procedures should be included to help outline the candidate’s experience, but won’t count towards the required number of cases. Minor procedures include: peripheral vascular cannulation, vascular access port implantation, castration, large reservoir subcutaneous pump implantation, and/or subcutaneous radio-telemetry device placement, etc. Examples of procedures that do not qualify as minor procedures are implantation of subcutaneous ID chips or any procedure using an injectable type device. Acting as a sterile assistant on major procedures may be accepted as long as the duties were significant (including at a minimum substantial dissection and closure) and are adequately described in the log. The case log must include the type of procedure, the date of the procedure, the species and sex involved, a record of any complications and their treatment, whether the procedure was performed aseptically, the final disposition of the animal, and the candidate’s role (primary surgeon or assistant). If the candidate’s role was as an assistant, the duties performed need to be described.
- **Narratives:** The applicant must submit two narratives of cases represented in the case log. Narratives should comprehensively describe all aspects of the procedure, including:
  - Drug regimens, including doses, routes of administration and timing related to the surgical procedure (prior, during, or after the procedure) for pre-anesthetic, analgesic, and antibiotic drugs
  - Justification for the regimen itself
The methods used to monitor the animal's condition and anesthetic depth pre-operatively, intra-operatively, and immediately post-operatively

Animal preparation for surgery including areas clipped, surgical positioning, and the antiseptic prep regimen

Detailed description of the surgical technique including incision creation, tissue dissection, identification of the tissues encountered, methods of hemostasis used, identification of critical instruments used, and closure techniques (suture patterns and materials used)

Acceptable survival surgical procedures involve a minimum of 72 hours of survival post-surgery before euthanasia

Post-operative monitoring and pain assessment including species appropriate signs of pain and distress and duration of monitoring and follow-up treatments

**Surgical Research Specialist (SRS) Certification**

- **Case Log:**
  - The SRS case log must have a minimum of 12 major, survival procedures in which the applicant acted as primary surgeon. There must be at least two different procedures, in two different species, other than a rodent. If procedures are performed only in rodents, a minimum of 24 cases must be included in the log with 4 different procedures being performed.
  - Major procedures include those that enter a body cavity, vascular anastomosis, significant orthopedic surgery, or involve significant CNS manipulation such as intra-thecal cannulation and nerve anastomosis.
  - The case log must include the type of procedure, the date of the procedure, the species and sex involved, a record of any complications and their treatment, whether the procedure was performed aseptically, the final disposition of the animal, and the candidate's role. If the animal is transferred to other personnel post-surgically, this observation may be included in lieu of reporting the final disposition of the animal.
  - Acceptable survival surgical procedures involve a minimum of 72 hours of survival post-surgery before euthanasia.
  - Non-survival procedures should be included to help outline the candidate's experience, but will not count towards the required total.

- **Narrative:** The narrative requirements for the SRS certification are the same as for the SRT certification.

For other questions concerning the process, e-mail the Certification Committee. Or visit the Academy's web site.

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Or another way to view the issue is: In 2012, the 4th Thursday in August is the 23rd. The fourth Thursday in August 2015 (the period for DeNovo review) is August 27th. Which means, that from August 23, 2015 through August 27, 2015, the PHS funded activity must be suspended and no PHS funds may be used for animal care or animal use. This calendar conundrum described occurs 5-6 times every year and has the potential for serious consequences for Duke research. Obviously this is a serious issue that requires an effective preventive methodology.

The prevention: While the NIH/PHS will authorize IACUC approvals for up to 36 months, going forward the Duke IACUC will approve protocols for 35 months and 3 weeks. This effectively pushes all DeNovo protocol reviews to the 35th month as the date that the continuing protocol must be reviewed and approved by the IACUC; a date that is within the PHS 36 month window. Protocols that are reviewed and approved during month 34 or 35 are continued without any issues or concerns, and without any periods of unapproved activity when using PHS funded grants.

How it works: At thirty-two months into the life of the protocol, the OAWA sends a Renewal Notice to the Principal Investigator advising them of the approaching deadline for a renewal protocol, and requesting that the renewal be submitted soon so that it can be reviewed during an IACUC meeting in the 34th or 35th month of the protocol.

If a protocol cannot be approved at the IACUC meeting on the 35th month, the Principal Investigator is advised that prior to the expiration date of the protocol (generally in 2–3 weeks after the 35th month meeting), all protocol live animals must be:

- Used and euthanized. No additional animals may be procured or new studies started until the new protocol is approved;
- Transferred to another of the PIs protocols; or
- Transferred to the DLAR Holding Protocol.

NOTE: Animals on the DLAR Holding Protocol remain the financial responsibility of the PI (e.g. per diem and all other animal care charges). No experimentation is authorized while on the DLAR Holding Protocol.

Yes, it seems a trifle matter of a few days. But the NIH doesn’t think so, and certain sister institutions have paid the price for allowing protocols to exceed 3 years to the day. It is the intent of the Duke animal program that this dysfunction not befall our research staff! Wishing you a wonderful research month.
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 laboratory. The form can be found on the OESO Laboratory Environment web page, under the “Lab Safety Audits and Onsite Evaluations” heading. Once completed, this form should be sent to OESO Laboratory Safety for approval (fax: 919.681.7509).

Because additional hazards exist in animal facilities, the Division of Laboratory Animal Resources (DLAR) further restricts access in all DLAR-Managed facilities. Persons under the age of 18 are not permitted in DLAR areas where laboratory animals are present or contact may occur. Minors who are Duke employees (i.e., summer interns) and have received a risk assessment by EOHW medical personnel and are listed as personnel on a Duke approved animal protocol are exempt from this DLAR prohibition.

Contact with laboratory animals means exposure to animal hair, dander and urine proteins, which can contribute to allergies. And were that not enough to worry about, there is also the potential for exposure to infectious diseases that can be transmitted from animals to humans.

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 animal facilities, all employees requiring access to animal facilities (including summer working minors) must complete the “Hazard Awareness for Animal Facilities” training on the OESO Safety Website (Training Modules).

Summer jobs and internships can be a great way for teens to gain some valuable science-related work experience. But minors in laboratories require additional protections. Let’s keep those developing young scientists safe in our laboratories!

If you are considering employing minors in animal facilities this summer, please contact OESO (919.684.2794) & DLAR (919.684.2797) for more information.