8th EDITION OF THE GUIDE REQUIRES POLICY ADJUSTMENTS
(Housing Density Policy)

The Duke IAUCUC recently updated the institution's housing density policy to make it fully complicate with the NIH's new 8th Edition of The Guide for the Care & Use of Laboratory Animals. Click the link in the title to go to the web version of the policy.

The Duke Policy balances The Guide recommendations against the style of caging and resources available at Duke where we have rodent caging ranging from 67 - 75 square inches. If cages are larger or smaller than 75 square inches, then The Guide recommendations shall be used, unless IACUC approval is received for the altered housing density. There are a few critical milestones to remember:

♦ Social Housing is the default.
♦ An adult mouse requires 15 sq. inches.
♦ For dams with neonates, numbers rather than body weight of the individual animals is a basis for assessing overcrowding.

There are always exceptions to every policy, and in this case; aggressive or breeding males (generally will be single housed), pregnant females in the last 1/3 of gestation (single housed to prevent parturition distress), and veterinary-ordered medical issues were part of the previous policy and have not changed. What did change is the need for IACUC approval of an exemption if more than one litter plus 2 adults is in the cage. Specifically, according to the space requirements specified in the Guide, having 2 litters in one of our standard-sized cages is overcrowded, even in the first few days after their birth.

For more information, visit the web policy or discuss the options with DLAR veterinarians or staff.

Requests for scientifically based exceptions may be submitted to the Duke IACUC using the email IACUC@duke.edu

Wishing you a wonderful research month,

POLICY REGARDING NOTIFICATION OF HAZARDOUS WORK IN ANIMAL CARE AREAS

The Duke IAUCUC recently updated the institution's Policy on Working With Hazardous Agents in Animal Care Areas to make the IACUC Policy and the DLAR Policy fully complicate.

For animals housed in DLAR-managed areas:

◊ At least five business days prior to dosing the animals, the laboratory must notify DLAR of the dosing schedule and send the Material Safety Data Sheet (MSDS) with a copy of the OESO-approved SOP to the DLAR Assistant Director, Head of Facility Operations & Operations Manager of the animal facility.

◊ When dosed animals are returned to their cages, hazardous agent cage cards (supplied by DLAR) must be placed on each cage, and the “toxic chemical hazard” sign (last page of this the OESO approved SOP) must be posted on the door (with hazard, dosing and contact information filled in).

◊ Cages occupied within the first 48 hours after dosing must be marked until the contents have been dumped.

◊ The cards and signs must be removed by the researcher once the hazard is no longer present (in most cases, after the first cage change that takes place more than 48 hours after animals receive their final dose).

Investigators can initiate work only after obtaining confirmation that notification has been received by DLAR.

For more detail and specific instructions, see the OESO website for hazardous agent in animal research.

The latest biomedical fiction ‘THRILLER’ from Paul McKellips (Vice President from the Foundation for Biomedical Research) is now available for order at http://jericho3.com/books/jericho-3/
If you enjoyed his first book ‘UNCAGED,’ you’ll love this one.

Proceeds from the sale of the book go to the FBR.
ZOONOSES
POTENTIAL BIOHAZARD ISSUES

Zoonoses—diseases that can be transmitted from animals to humans. May be found in nearly all animal species.

Potential biohazard issues: Although the risk of transmitting zoonotic disease in an animal facility is fairly small, it should be understood that nearly all animal species are known to be a host of at least one zoonotic agent. Quality veterinary care programs, proper quarantine of new arrivals and the purchase of animals from reputable suppliers significantly reduces the risk of such agents from entering animal facilities.

So, how do YOU limit your potential exposure to zoonotic diseases while working with animals at Duke? Don’t know? Ask a DLAR Staff member! They know.

ALLERGEN EXPOSURE

Perhaps the most common health concern in research animal facilities, allergen exposure depends on parameters such as animal species, ventilation system, work practices and the employees “Health screen”.

Allergic reactions to animals are among the most common conditions that adversely affect the health of workers involved in the care and use of animals in research. All animal handlers (Duke employees, students or other personnel) must complete the Employee Occupational Health and Wellness (EOHW) Placement “Health Review for Animal Handlers” prior to the start of work with animals and a Periodic Health Review for Animal Handlers form at least annually thereafter.

How can you Duke control your exposure to allergens? Use:

- Ventilated caging & bedding dump stations
- Biological Safety Cabinets
- HEPA-filtered Change Stations
- Gloves
- Gowns
- Shoe Covers
- Hair Bonnets (Bouffant Caps)
- Masks - Respirators**
- Face Shields
- PAPR**

*Note - Specific training from OESO is required for the wearing of a respirator (N-95) or PAPR device.

THE FOUR R’S REVISITED

One of the primary components of protocol development is inclusion of the 4 R’s (Refinement, Replacement, Reduction, and Responsibility). Here are a few reminders from the 8th edition of The Guide for the Care and Use of Laboratory Animals.

- **Replacement and Reduction** goals should be balanced on a case-by-case basis. In other words, reduction should not serve as rationale for reusing animals that have already undergone experimental procedures especially if the well-being of the animals would be compromised.
- **Refinement** refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. While institutions and investigators should take all reasonable measures to eliminate pain and distress through refinement, IACUCs should understand that with some types of studies, there could be either unforeseen or intended experimental outcomes that produce pain. These outcomes may or may not be eliminated based on the goals of the study.
- **Responsible** use of animals requires recognition that they are sentient and feel pain. Use of animals in research is a privilege granted by society to the research community with the expectation that such use will provide either significant new knowledge or lead to improvement in human and/or animal well-being. This trust mandates responsible use with each animal every day.

Pro-Research Initiatives Trump Lackluster Animal Rights Protests During "World Week" for Laboratory Animals

It has happened every April for the past two decades plus, a week designated by animal rights groups to draw media attention to the issue of the use of laboratory animals in biomedical research. At one time, 'The Week' could count on hundreds to show up for scheduled protests at research institutions. Not this year.

While over a score of demonstrations were announced for April 19-26, most drew fewer than a dozen people each, while some planned protests never materialized. The largest demonstration in the U.S. seems to have been on the campus of the University of California, Los Angeles, where about 50 protesters paraded for a few hours last week.
REPORTING MISSING or ESCAPED ANIMALS

Even under the most controlled circumstances, adverse events may occur. When an animal escapes the holding cage, it is important that measures to capture the animal are engaged. It is not acceptable to consider it ‘returned to the wild’ or ‘free to roam the building.’ The goals of recapture are to: a) prevent an injury to an animal unaccustomed to the out-of-doors; b) to prevent a transgenic animal from passing their modified genes to other animals; and c) to prevent the spread of potential pathogens (if the animal is infectious).

According to Duke animal care program polices, all personnel who work with animals must be trained in handling, restraint, and capture of animals.

The responsibility for ensuring appropriate training of the research staff lies with the PI (for PI managed spaces) and with DLAR (for DLAR managed spaces).

Select considerations for recapturing animals include:

- A rodent which has escaped should not be handled by hand. Use a hard container (e.g., a cup or empty cage) when capturing 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 the responsible PI can be determined, they will be notified immediately.
- If you suspect an animal is missing, check the room mortality log to see if an animal has died and the carcass removed for refrigerated storage.
- If you cannot determine that an animal is missing, or you know it is missing and cannot find the remains, then contact the DLAR supervisor.

It is especially important to notify DLAR management if the missing animal is a transgenic animal, 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.

STANDARD OPERATING PROCEDURE REQUIREMENTS FOR ABSL2 CONTAINMENT AT DUKE

The Principal Investigator (PI) has the responsibility to inform the laboratory personnel of the appropriate research procedures. When using hazardous or regulated biological agents the PI must prepare a written Standard Operating Procedure (SOP) outlining the necessary precautions to safely conduct research. An SOP is a set of specific guidelines designed to address the methods that will be used and the safe handling of biological agents. The SOP must be available in the laboratory.

The SOP is a valuable tool and worth the preparation time. A well-written SOP can be used to satisfy several compliance requirements. SOP should be written for all procedures that pose an identified potential risk to the health and safety of the laboratory personnel, although a separate SOP does not need to be written for each individual experiment, procedures with the same hazards can be combined into one SOP.

The process of writing SOPs requires an individual to think through all steps of a procedure and perform a risk assessment before work has begun. The best approach to writing an SOP is to do it, write it and test it. Be brief and succinct; the shorter the better. A SOP template is available on OESO Biosafety Web Site: [http://www.safety.duke.edu/BioSafety/Animals.htm](http://www.safety.duke.edu/BioSafety/Animals.htm)

OESO HAS SEVERAL GUIDELINES FOR SOP DEVELOPMENT OF HAZARDOUS AGENT USE IN ANIMAL PROTOCOLS

OESO Biosafety Division has a great web site which assists researchers with specific SOP development! For example:

- Guide for Developing an SOP for the use of Biohazards in Animals
- Guide for Developing SOP for the use of Hazardous Drugs
- SOP for the use of Toxic Chemicals in Animals
- Guidelines for the Safe Handling of Animals Exposed to LPS in Research
- Radiation Safety Animal Care and Use Protocol Wizard

You can reach this site and use this links by going to the OESO Biosafety site at: [http://www.safety.duke.edu/BioSafety/Animals.htm](http://www.safety.duke.edu/BioSafety/Animals.htm)

Upcoming Dates & Deadlines

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

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<tr>
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<tr>
<td>June 4</td>
<td>New Protocol Deadline</td>
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<td>June 7</td>
<td>Amendment Meeting</td>
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<td>June 11</td>
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<td>June 21</td>
<td>Amendment Meeting</td>
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<td>June 25</td>
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<td>June 28</td>
<td>New Protocol Meeting</td>
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As a research community, we have effectively turned the tide with regard to public support for the humane and responsible use of animals in research, at least here in the United States...and at least for now.

Zogby International polled 2,102 adults (April 26-30th) with a margin of error of 2.2%. Exactly 60% of those polled supported the use of animals in research. Among men (1,015 participated) support stood at 74.4% and among women (1,078 participated) support hit 58.1%.

Support among Democrats was 62.5%, Republicans registered 70.7% support and Independents tallied 65.1%. Regionally, the strongest support was in the Great Lakes states (69.4%) and the East was the least with 61.0%.

Research!America has started Speaking up! for Science, a website designed to encourage scientists and especially science students to get involved in advocacy. The first focus of this new initiative was a letter to Congressional leaders urging them to assign a high priority to medical research funding.
CONTROLLED SUBSTANCE USE AND MANAGEMENT PROCEDURES

Biomedical research, testing, and teaching programs involving animals often require controlled substances for prevention of pain or distress. Controlled substances are used to produce anesthesia, analgesia, tranquillization, sedation, hypnosis, euthanasia, or as a means to study the actions of specified drug regimens. Controlled substances have the potential for misuse. Accordingly, the federal agencies (DEA) and state offices (DHHS) require individuals using controlled substances to obtain both state and federal registrations, and to abide by the guidance describing licensing, storage, distribution, use, and disposal of controlled substances.

Registration for use of controlled substances is an individual obligation – there is no statutory requirement for institutional management of controlled substance registration. Even so, Duke University has an interest in the health and well-being of its animals and regulatory compliance of its researchers, employees, faculty, and staff. Therefore, Duke University has appointed the Duke Office of Animal Welfare Assurance (OAWA) to assist controlled substance registrants by providing guidance and oversight, which will help assure compliance with all federal regulations, state rules, and institutional requirements.

The Duke Institutional Animal Care & Use Committee (IACUC), OAWA, and the Division of Laboratory Animal Resources (DLAR) have established mutually supportive procedures for local use of controlled substances in animals under Duke approved research protocols. These procedures have been accepted as supporting the goals of the state and the federal agencies.

These procedures apply to those situations where controlled substances are being used for animal anesthesia, analgesia, euthanasia, restraint, or experimentation under the auspices of the Duke University animal care & use program. All controlled substance use must be in accordance with the IACUC-approved protocol, or under accepted clinical veterinary guidelines as prescribed by a Duke veterinarian.

Failure to abide by federal and state controlled substance regulations may serve as a basis for suspension or termination of the affected animal research protocol by the Duke IACUC. Suspension or termination of a protocol based upon controlled substances diversion shall also result in a report to Duke compliance officials, and notification of the state and federal agencies.

The controlled substance registrant must hold both North Carolina Department of Health & Human Services (DHHS) and federal Drug Enforcement Agency (DEA) registrations. You may not place an order, store, or dispense controlled substances without both registrations. For instructions to apply for a registration, click here.

Other links which may be useful include:

⇒ Controlled Substance Brown Bag Seminar
⇒ Controlled Substance Forms (Excel version)
⇒ Example of Properly Completed Purchase Log
⇒ Example of Properly Completed Use Log
⇒ Example of Mixture Use Log
⇒ Example of Physical Inventory Log
⇒ Purchase (or transfer) Log Sheet
⇒ Use Log (Individual Drug Tracking)
⇒ Use Log (Combination *Mixture* Drug Tracking)
⇒ Quarterly Physical Inventory
⇒ Sample IR for DLAR purchase
⇒ Authorized Users List
⇒ Completed example of DEA form 222
⇒ DEA Form 225: Federal C. S. Application
⇒ DEA Controlled Substance Code Numbers
⇒ NC DHHS Form 225: NC State Controlled Substance License Initial Application
⇒ NC State Instructions for DHHS 225
⇒ NC DHHS Annual Renewal Letter (Example)

Questions? Call OAWA at 919.668.6720
OLAW Q&As
Regarding Non-Pharmaceutical Use of Agents in Animal Research

In January 2012, the NIH’s Office of Laboratory Animal Welfare accepted the 8th edition of The Guide for the Care & Use of Laboratory Animals as a required reference for PHS Assured institutions. Duke is PHS Assured, which means we are required to abide by the precepts of The Guide, if we are to receive NIH/PHS funding for research activities. PHS Assurance is a necessity for our access our than NIH funded activity also.

Several items discussed in the 8th edition have affected Duke research, most have been minimal, but a few have substantially changed the practice and procedures of animal care and animal use. One of the more discussed issues has been the new stipulations regarding the use of non-pharmaceutical agents in animals. While the NIH’s position can be traced back to 1993, the publication of the 8th Edition has galvanized the NIH’s long-standing position, and the NIH has established a more clear position on the use of non-pharmaceutical agents in federally funded research.

The Health Research Extension Act of 1985 is the statute that confers the authority to oversee PHS-supported animal activities to NIH. The Congressional Committee report that accompanied the law stated, “It is far preferable to place primary responsibility for assuring compliance with NIH guidelines on committees within institutions rather than relying on intrusive Federal inspections.” OLAW interprets this to mean that Congress intended for IACUCs to have the authority and the responsibility to make meaningful decisions about biomedical research conducted with animal models. The PHS Policy (IV.C.1.a.-g.) restates the concept. “In order to approve proposed research projects… the IACUC shall conduct a review of those components related to the care and use of animals and determine that the proposed research projects are in accordance with this [PHS] Policy.

The next few pages provide Q&As from the NIH regarding specific issues or practices involving non-pharmaceutical agents.

Question: How does one determine whether a particular drug is available in a pharmaceutical-grade? Is there one place to determine what the source(s) might be?

Answer: You may determine what is available by consulting the FDA database. The Orange Book is the reference for FDA-approved human drugs. The Green Book is the reference for FDA-approved veterinary drugs.

Question: Can diluted Fatal Plus (an agent commonly approved for euthanasia) be used for perfusions?

Answer: The use of Fatal-Plus for anesthesia is specifically prohibited by FDA in the instructions included on the label of the product. The extra-label use of a euthanasia product for its pentobarbital content is unacceptable and violates the PHS Policy and Animal Welfare Act and Regulations. Such proposed use may not be approved by an IACUC or used by investigators at Assured institutions or used on regulated species. We note that some euthanasia procedures include perfusion of the animal prior to death. FDA approved euthanasia solutions may be used in those procedures in combination with the perfusion agent to perform perfusion and euthanasia as a single procedure.

Question: Must investigators identify any drugs, biologics, or reagents that will be administered to animals in IACUC applications? For example, eye ointment, fluids, heparin, antibiotics, atropine, mannitol, tattoo ink, extract of plant root, biological material that has just been identified such as a gene, siRNA, or protein, or a newly synthesized chemical compound? Must each of these be justified to the IACUC?

Answer: Any drugs, biologics, or reagents that are administered to animals as part of a study or experiment must be included on the animal study proposal and reviewed and approved by the IACUC. Drugs or substances used in clinical practice on the research animals must be pharmaceutical-grade, if possible, and do not have to be listed in the animal study proposal. It is up to the IACUC, practicing local self-monitoring, to ensure that pain and distress are avoided or minimized, while at the same time supporting justified scientific research. The system of protocol evaluation allows the IACUC and investigators to work together to determine an optimal way to conduct experiments within the context of humane animal care. OLAW and the Guide have repeatedly stressed that investigators and IACUCs are empowered to use professional judgment.
Question: Can chemical-grade tamoxifen mixed in oil; chemical-grade doxycycline; or non-pharmaceutical-grade PMSG be used in research animals?

Answer: While it is not possible to answer the question for every situation, the NIH/OLAW has stated on several occasions that it would be reasonable for the IACUC to review and approve the use of non-pharmaceutical-grade substances under the following circumstances:

1. If no equivalent veterinary or human drug is available for experimental use, then the highest-grade equivalent chemical reagent should be used and formulated aseptically and with a non-toxic vehicle as appropriate for the route of administration.

2. Although an equivalent veterinary or human drug is available for experimental use, the chemical-grade reagent is required to replicate methods from previous studies because results are directly compared to those of replicated studies.

3. Although an equivalent veterinary or human drug is available, dilution or change in formulation is required. Furthermore:
   • If adulteration by dilution, addition, or other change in formulation is required, there may be no additional advantage to be gained by using the USP formulation.
   • Use of the highest-grade reagent may have the advantage of single-stage formulation and also result in purity that is equal to or higher than the human or veterinary drug.
   • Professional judgment should be used to determine the appropriate test material and to ensure use of an agent with the least likelihood for causing adverse effects.

4. The available human or veterinary drug is not sufficiently concentrated to meet experimental requirements.

5. The available human or veterinary drug does not meet the non-toxic vehicle requirements for the specified route of injection.

However, even if the above circumstances existed, the IACUC must still consider factors including, for example, the grade, purity, sterility and acid-base balance, pyrogenicity, osmolality and stability, the site and route of administration, compatibility of components, side effects and adverse reactions, storage and pharmacokinetics.

Continued Next Column...

Question: To what extent does NIH/OLAW expect IACUC’s to evaluate unusual vehicles (e.g., Cremophor, DMSO, DMF, PEG400) which may be required to get the compounds into solution?

Answer: Vehicles administered to animals in biomedical research must be pharmaceutical-grade, if available. Use of non-pharmaceutical-grade vehicles must be justified and that justification must be reviewed and approved by the IACUC. The IACUC and investigator must consider the route of administration; products administered orally should be food-grade.

Question: Many of the drugs that we use do not say if they are pharmaceutical-grade. Many of these are ordered from Sigma. We cannot find a reliable method for determining if these are or are not pharmaceutical-grade. Can you tell us a place or way that we can find that information on MSDS, website?

Answer: OLAW provides the following definition (Position Statement 3): A pharmaceutical-grade compound is a drug, biologic, or reagent that is approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary (USP-NF), or British Pharmacopeia. If the product does not meet these standards, it is not a pharmaceutical-grade product. Reagents are not drugs. Drugs are manufactured by a pharmaceutical producer under good manufacturing practices and approved by the FDA. OLAW recommends that the investigator contact the manufacturer, if the information cannot be found on a commercial website. Also, the Green Book (veterinary) and the Orange Book (human) available on the FDA website, provide a list of FDA-approved, pharmaceutical-grade drugs.

Question: Some investigators use chemicals from SIGMA for various compounds (e.g., tribromoethanol) for anesthetics that are not made in a pharmaceutical grade. However these chemical bottles have no expiration date labeled. They appear to have a date of manufacture only. I do not know how long these chemicals can be expected to be "in date". Do you have any guidance on this?

Answer: If you cannot find information that you need about a product on a commercial website, contact the manufacturer. It would be reasonable for the IACUC to ask the investigator to provide information about stability of a compound prepared in the laboratory. OLAW Position Statement 3 states: “The IACUC is responsible for evaluating the potential adverse consequences of such agents when used for research. In making its evaluation, the IACUC may consider factors including, for example: grade, purity, sterility, acid-base balance, pyrogenicity, osmolality, stability, site and route of administration, compatibility of components, side effects and adverse reactions, storage and pharmacokinetics.”

Continued on the Next Page...
Question: Are Sigma USP reagent offerings considered pharmaceutical-grade (such that no special justification is required on a protocol to use them in animals)?

Answer: No. Reagents are not pharmaceutical-grade. Use of reagents must be scientifically justified. The institution may develop an institution-wide policy on the use of reagents.

Question: How do non-pharmaceutical grade requirements apply to diluents used in drug discovery? What is OLAW’s stance on the use of pharmaceutical-grade substances infused within diets?

Answer: It depends on the route of administration and the need to maintain sterility. OLAW’s concern is that the substance doesn’t injure the animals and is appropriate for the science. Professional judgment should be used in making this determination. For oral administration, the vehicle or diluent should be food grade. For injections such as intramuscular, intraperitoneal, or subcutaneous, the diluent or vehicle should be sterile and physiologic.

Question: Please clarify whether this guidance only includes medical/veterinary substances or if it also included substances that are used in the vehicle for test and control articles.

Answer: USDA and OLAW agree substances administered to research animal subjects must be pharmaceutical-grade unless scientifically justified. This applies to research, medical and veterinary substances and vehicles for test and control substances. For many test or novel agents, a pharmaceutical-grade may not exist so the IACUC needs to approve this use based on scientific justification. The PI should give as much information as possible about any adverse reactions of the proposed class of agents.

Question: If I generate antibodies from a rabbit, will I be able to use them in my studies?

Answer: This decision will be up to the IACUC. The proposed study should be reviewed by the IACUC in the same way that any proposed study would be. There is no overriding prohibition against using antibodies that were generated in an animal in such studies. However, antibodies should be made in vitro, rather than in vivo, if possible.

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Question: Do we need to use pharmaceutical-grade sterile saline as a diluent for anesthetics or other drugs? Some drugs require sterile water or other chemicals to make up the diluents. Do all solutions/chemicals put in the diluents need to be of pharmaceutical-grade?

Answer: OLAW considers the compounding of investigational agents or the customized manipulation by dilution or addition of vehicles to pharmaceutical-grade substances for administration to animals as necessary and acceptable scientific activities carried out by researchers in their laboratory. However, these activities should be described in the animal study and reviewed and approved by the IACUC using the same criteria as previously discussed. The diluent requirement is the same as the primary agent, that is, pharmaceutical-grade and sterile unless an alternative is needed based on scientific justification and approval by IACUC.

**Animal Tracks**

**Name the Procedure**

Question: What is happening to this animal?

Answer: Application of ophthalmic ointment to the cornea. Anesthesia or sedation causes a decrease in blinking and usually a decrease in tear production. If you have ever had a dry eye or a speck of dirt in your eye, you know how much it hurts! To prevent your research animals from being in eye pain, always protect the corneas by using an ophthalmic ointment. It is not so critical what type of ophthalmic ointment is used (e.g., antibiotic or simple lubricant), but the preferred ointment should not have a steroid in it. Always avoid touching the eye with the tip of the ointment dispenser as it may scratch the cornea. It is also important to double check the ointment and be sure it is approved for use in the eye. There are many types of ointments, but only those that state ‘FOR OPHTHALMIC USE’ should be used to protect the animal’s eye.

If you have questions, just call the DLAR or OAWA. Don’t forget: Animal welfare is EVERYONE’s obligation!