You have a strong scientific idea that really needs to be tested using animals – but you can’t seem to get the IACUC to approve your proposal for animal use. Why? Are the regulators finally taking control of science and stifling good research? Are animal rights advocates making the reviewers so frightened that your idea can not get an fair review? Is the Committee so dense that it cannot see the merits of your proposal? The answer to all of the above is ‘FALSE.’ It really isn’t difficult to get a protocol approved by the IACUC … if you know the ‘secrets.’ Let’s see if I can cut through some of the mystery and help with your next protocol submission.

1st Secret: Lay Language (B1: Purpose): This section of the protocol CANNOT be written in scientific language, and cannot have a myriad of acronyms! Some researchers miss the instructions for this section (included on the form) that state “Describe in lay terms the purpose of this animal use study.” Do not write section B1 as if they applying for a grant. Using technical or highly scientific language for this section will delay approval. This is not an IACUC requirement, but rather a requirement of the United States Department of Agriculture, that the IACUC must enforce. Federal guidance requires that certain aspects of the protocol MUST be written in lay language, or language that is understandable by the general public - another way of defining lay language is to consider it as 'newspaper' language, or ‘senior high school’ level English. Do not use scientific or highly technical terms that one would not find in a newspaper article. An example of ‘lay language’ can be found at http://vetmed.duhs.duke.edu/guidelines_for_writing_good_lay%20descriptions.htm

2nd Secret: Justifications:
   (B3: Alternatives to Painful Procedures): The USDA has determined that any use of analgesics, anesthetics, or tranquillizers require that alternatives to painful procedures be considered. If selecting ‘YES,’ then a literature search for alternatives to the proposed procedures are necessary. The animal program web site has information to assist with assuring the IACUC that an appropriate search has been performed.
   (B5: Animal Justification): The Animal Welfare Act requires that the principal investigator provide assurance that alternatives to the use of live vertebrate animals were considered in planning these research activities. The proposal must include a justification with sufficient detail to assure the IACUC that alternative means of achieving the research goal were considered.

Researchers and associates in our Duke research community are concerned about, and work diligently to prevent, pain and distress. The problem for most of us is recognizing the signs of pain or distress in animals. Animals express pain or distress using different behaviors than humans and sometimes we miss what our animals are trying to tell us. Animals tend to ‘hide’ outward signs of pain which further complicates our ability to tell when they are suffering. Most of our research species hide signs of discomfort as long as possible. Knowing signs of pain in animals is critical to a pain management plan. Observing one or more signs of pain can tell us when our pain management plan needs enhancement.

Part 1 was presented in the December 2007 issue of Animal Tracks and covered the most common research animals—mice and rats. In this issue we will look at other species used in research.

Nonhuman Primates (NHP): Monkeys often show remarkably little reaction to surgical procedures or to traumatic injury. Obvious signs of pain are not readily seen. Loud and persistent vocalization, for example, commonly signifies only alarm or anger. The animal in pain may be huddled in a crouching posture with a "sad" facial expression and glassy eyes, or it may sit hunched with its head forward and its arms across its body. It may avoid its companions and may stop grooming itself. A monkey in pain may also attract increased attention from its cage mates, which can vary from social grooming to attack.

(Continued on the next page)
Acute abdominal pain may be shown by facial contortions, clenching of the teeth, restlessness, and shaking accompanied by grunts and moans. Food and water intake is usually diminished or absent. Key Signs: hunched position, failure to groom, refusal of food or water, dejected appearance.

Guinea Pigs: Guinea pigs are alert, but timid and apprehensive animals which will try to avoid capture and restraint. Rarely is there any aggression towards humans. Any sign of acceptance indicates the animal is unwell. Loud vocalization will accompany even minor and transient pain. Guinea pigs often appear sleepy when in pain. Initially, there is an increased level of response to painful or stressful stimuli. However, this gradually subsides and the animal becomes unresponsive. It gradually appears more apprehensive. The eyes may be sunken and dull. The respiratory rate increases as a painful or stressful stimulus increases or continues; where the respiratory system is affected, respirations become increasingly forced and labored. Often loss of weight occurs as well as hair loss, scaly skin, and dehydration. Where the gastrointestinal tract is affected there may be evidence of diarrhea. There is a tendency to 'barbering' under dietary stress with failure to eat or drink. Group aggression may occur and damage to the skin of the back may result from fighting. There is excessive salivation where abnormal teeth cause eating difficulties, a tendency to an arched back where abdominal pain is present, and failure of the "righting" reflex in seriously ill animals. There may be pain associated with locomotion, lameness, and careful gait due to sore feet in older animals. Key Signs: withdrawal, vocalization, failure to resist restraint, unresponsive.

Mongolian Gerbils: Gerbils are highly active, nervous animals and usually attempt to avoid restraint. Signs of pain and distress are difficult to assess, as gerbils apparently object to any interference. There is an increased level of response under painful or stressful stimuli. Ocular discharge is common. Under stressful conditions, the eyelids may be half closed, with dry matting of the eyelids. The increased respiratory rate associated with lung involvement is difficult to assess by eye. Loss of coat condition occurs. Loss of hair from the tail may be seen in overcrowded animals. Facial lesions and sores may result from excessive burrowing in the corners of the cage. Dehydration is rarely seen, since the gerbil's normal metabolism enables full utilization of the water content of the diet. Only small quantities of urine are voided under normal conditions. Feces are normally firm, dry pellets. Constipation is rare. Diarrhea, if it occurs, may quickly lead to death from fluid loss. Gerbils are normally extremely active and nervous. Under severe stress, there may be temporary collapse and apparent shock syndrome; however, the animals recover, given time. Changes in exploratory behavior and increased aggressive response may occur. A hunching up and arching of the back may be observed, especially with abdominal involvement. Abnormal gait is associated with locomotion or abdominal involvement. Key Signs: hunched appearance, weight loss, shock syndrome.

Hamsters (Golden or Syrian): Under normal conditions, hamsters will sleep for long periods during the day, and little activity will be seen. They often appear aggressive towards their cage mates and emit loud screeching noises, disproportionate to the degree of interference, when handled. This response increases under painful or stressful stimuli. Ocular discharge is commonly associated with stress. An increased respiratory rate is associated with lung involvement. Loss of coat condition is seen where the diet is deficient in Vitamin E and short chain fatty acids. Loss of body condition occurs with decreased food and water intake. Constipation is unusual in the hamster. Diarrhea, when it occurs, is profuse and liquid, staining the perineal region. Increasing depression takes place when the animal is left undisturbed. Daytime sleep periods may be extended and increasing lassitude may be seen except when the animal is being handled. Exploratory behavior is reduced. A hunched appearance is noted, as is an unwillingness to move, especially where abdominal organs are involved. Lateral recumbency can indicate that the animal is moribund. Normal gait is affected when pain is associated with locomotion. Stilted movements are sometimes associated with abdominal involvement, e.g., ascites following cirrhosis of the liver. Key Signs: weight loss, hunched appearance, increased aggression or depression, extended sleep periods.

Rabbits: The rabbit presents significant difficulties in recognition of pain and distress, as it often quietly accepts apparently painful or distressing procedures; this may relate to its feral behavior where concealment is important to survival. Even healthy rabbits may not move frequently or indulge in exploratory behavior. Pain is usually characterized by a reduction in food and water intake (and thus weight loss and dehydration) and limited movement. Although rabbits frequently become ill and distressed without showing much apparent loss of condition, careful examination will reveal a loss of muscle mass on the lower back. Ocular discharge is a common response to stress in the rabbit, with protrusion of the nictitating membrane. Under continued pain or stress, rabbits assume a 'sleepy' appearance. The animal exhibits increased depression, progressive unawareness and lack of response. The animal will often face the back of cage, away from light. An increased respiratory rate is associated with either apprehension or lung involvement. There is fecal staining of the coat. Night time pellet production may be interrupted. Constipation and diarrhea are common responses to pain or stress. Excessive self-grooming may precipitate hair balls in the stomach. Where foot soreness is involved, weight may be thrown forward or backward to reduce discomfort. Body stretching and lying flat are common indications of abdominal discomfort. Pain may be associated with locomotion, especially with sore feet. Key Signs: reduced eating and drinking, faces towards back of cage, limited movement, and apparent photosensitivity.

Next month’s issue will contain part 3 which will cover sheep, goats, pigs, birds, reptiles, and fishes.
Species Justification (B6): An important requirement for an animal use application is a clear and accurate argument for the use of the species required for the proposed study. Why can’t the proposed activities be done using non-animal models, computers, or carcasses? Why can’t rodents be used instead of swine? A good example of an effective species justification can be found at [http://vetmed.duhs.duke.edu/guidelines_for_writing_good_species_justifications.htm](http://vetmed.duhs.duke.edu/guidelines_for_writing_good_species_justifications.htm).

Animal Numbers Justification (B7): While the funding grant only requires estimated numbers of animals, federal guidance prohibits the IACUC from approving estimated numbers. The IACUC can only approve a finite maximum number of animals for a specific purpose. Sometimes this is not an easy task, but there is no other option. Some proposals will require statistical significance, while other proposals will justify numbers based upon required tissue quantity, training objectives, or population dynamics (e.g. field study). The animal program web site has a primer on designing a justification for animal numbers. Visit the url [http://vetmed.duhs.duke.edu/documents/reference/statistics_primer_for_lab_animal_researchers.pdf](http://vetmed.duhs.duke.edu/documents/reference/statistics_primer_for_lab_animal_researchers.pdf) for more details. Options are included in the protocol template.

3rd Secret: Personnel Training: IACUC approval is sometimes delayed because of personnel training issues. All individuals listed on a protocol with animal handling responsibilities, must complete the two part Animal Handler Training modules on the OESO web site. If your protocol proposes CO2 as a method of euthanasia, all personnel must complete the online CO2 training. Failure to complete these training requirements will delay IACUC approval.

4th Secret: Occupational and Environmental Safety (OESO): Duke requires that all people at Duke work in an environment that is safe and healthy. OESO reviews all animal protocols and manages the safety aspects on animal use on campus. In certain cases, a further review by the Institutional Biosafety Committee (IBC) may be required prior to granting safety ‘clearance’ for animal work. Clearance from OESO is required before the IACUC can grant protocol approval. It is always a good idea to discuss concerns or problems with Dr. Joan Catignani (joan.catignani@duke.edu) before submitting your protocol for the IACUC’s review.

5th Secret: Employee Occupational Health & Wellness (EOHW): Duke requires that all people at Duke receive adequate healthcare coverage and are sufficient healthy to work with animals. EOHW provides this oversight service to the research community. Clearance from EOHW is required before the IACUC can grant protocol approval. You can submit the required information using the form: "Placement Health Review for Animal Handlers" on the animal program website. Specific concerns or problems can be discussed with Terri Weddle (EM: wedd001@mc.duke.edu; Ph: 681.0555) or Carol Epling (EM: eplin002@mc.duke.edu; Ph: 684.4651).

6th Secret: Signatures are not required: If the protocol, amendment, or personnel data forms are sent from the PI’s Duke Email address, signatures are not required. If any other means of submission is used, all documents require the signature of the PI or the affected person. Failure to use the PI’s Duke email account or have the signatures for forms sent in other manners, will prevent the IACUC from granting the approval – even if all other aspects of the protocol process have been completed.

7th Secret: Respond promptly: If you receive an email concerning the administrative review, the veterinary pre-review, or the IACUC member review, please respond promptly. The review process follows a strict time line, because the IACUC must have a complete review package. The IACUC is prohibited from approving proposals for which there is an incomplete information file. Since the IACUC meets only once a month, if timely approval is important to the research initiative, then respond as quickly as possible to emails from reviewers or administrative support staff. At any time, and if you need more ‘secrets’ of IACUC approval, please call the Office of Animal Welfare Assurance (668.6720). Our staff are always ready to facilitate your research needs at Duke. Wishing you a safe and productive research month,
GETTING STARTED:

⇒ VISIT THE DUKE ANIMAL PROGRAM WEB SITE at: http://vetmed.duhs.duke.edu/
  ◊ Familiarize yourself with this web site. This is the principle source for animal program forms, policies, guidelines, directions for training, or process procedures used in the Duke animal care and use program.
  ◊ For more specific protocol development guidance click on the link at the left titled “Protocol Development.” You may access the protocol template from this page, or by using the next step.
  ◊ The protocol template may be found on the page (http://vetmed.duhs.duke.edu/index_of_new_protocol.htm).
    ◊ Review the protocol template instructions.
    ◊ Download the appropriate version on the template for your animal protocol (most researchers select ‘Option A’).
  ◊ Visit the ‘IACUC Meeting Deadlines’ web page to confirm the review and approval schedule that best meets your needs (http://vetmed.duhs.duke.edu/index_of_iacuc_meeting_deadlines.htm). While most protocols are approved at the first IACUC meeting, you should plan on a 2 month approval process, just to be safe.

⇒ OBTAIN YOUR DUKE ‘NetID’ AND ‘Password’: While the animal program site is freely accessible, you will need your NetID and Password for certain steps toward protocol approval. NetID and Passwords are automatically created when you initiate a relationship with Duke (e.g. grad students’ fees are paid at the registrar; faculty position acceptance letter has been received by Duke HR). If you have problems or don’t know your NetID or Password, call the Office of Information Technology (OIT) at 919.684.2200.

⇒ COMPLETE THE HEALTH REVIEW: The web form for ‘Health Review for Animal Handlers’ is located at: https://www.hr.duke.edu/secure/eohw/animal.php. Once submitted, the assessment usually takes a few days, but may take longer if EOHW (Employee Health) determines additional medical assessment or immunizations are necessary prior to working with animals, animal tissues, or animal byproducts.

⇒ COMPLETE THE BASIC WEB TRAINING FOR ANIMAL USERS: Visit the OESO (safety office) web site at: http://www.safety.duke.edu/. Select the link on the left ‘On-line Training.’ Enter your NetID and Password. Select ‘Courses Available On-Line.’ All animal users must complete Animal Handlers I and Animal Handlers II. If you are working with rodents and will use CO2 for euthanasia, you must also complete ‘CO2 Euthanasia of Rodents.’ You may complete any of the other courses you feel are appropriate for your lab procedures. The IACUC, OESO, or EOHW may require specified web courses (e.g. biosafety work may require completion of the biosafety web module) during the protocol review.

⇒ FACILITY ORIENTATION: Access to animal vivaria requires an approved protocol and Facility Orientation. Contact Peg Hogan, RLATG, CMAR (ph: 919.684.3885) or Email hogan012@mc.duke.edu for scheduling.

(continued on next page)

QUESTIONS, CONCERNS, OR COMPLAINTS ABOUT THE CARE AND/OR WELFARE OF RESEARCH ANIMALS?

Please contact the Duke Animal Welfare Hotline (919.684.3535) or Email the Duke IACUC @ IACUC.Duke.edu

Any individual who has concerns related to the use of animals in biomedical research at Duke University is encouraged to voice those concerns. Duke University will not tolerate any reprisal against an individual who has come forward with concerns or allegations of wrongdoing involving the care and use of animals. Such reprisal is prohibited by federal law (USDA Regulations & the 9th Code of Federal Regulations). Individuals who feel that a personnel action has been taken against them because they reported an apparent violation of animal care and use requirements, should present their case to Chairman of the IACUC or the Director of the Office of Animal Welfare Assurance.

James Reynolds, Ph.D.                                      Ron Banks, DVM                                      John Norton, DVM, PhD
Chairman, IACUC                                            Director, OAWA                                      Director, DLAR
919.684.6720                                                919.684.4744                                        919.684.4204
IACUC@Duke.edu                                              ron.banks@duke.edu                                  john.norton@duke.edu
PROTOCOL SUBMISSION:

⇒ PRE-REVIEW: To assist researchers with protocol development, the Office of Animal Welfare Assurance (OAWA) provides a pre-review service. Just complete your application for animal use as best you can, and Email it to IACUC@DUKE.EDU. An OAWA veterinarian (Dr. Banks, Sharp, or Vanderford) will review your application and offer suggestions for improvement, in the hopes of achieving IACUC approval on the first review.

⇒ SUBMISSION FOR IACUC REVIEW: When ready, submit all applications for animal use to IACUC@DUKE.EDU. Once an application is submitted, DO NOT revise the document; only reply to the Emailed questions. Emails become part of the protocol file. A revised application will require re-review and may delay protocol approval.

◊ ADMINISTRATIVE REVIEW: You will receive a confirmation from one of OAWA’s Protocol Specialists that your protocol has been added to the IACUC’s agenda. The Protocol Specialist will also perform an ‘Administrative Review’ and may suggest enhancements (e.g. missing contact information, missing housing or procedure location, etc.). Please reply by Email with the information requested.

◊ VETERINARY REVIEW: All protocols receive a review by a Duke veterinarian prior to going to the IACUC. This review will include the entire protocol, but will focus on animal care, anesthesia, analgesia, and other animal care activities. The reviewer may identify specific concerns and offer suggestions to reconcile the concern. Please reply to the Email with the information requested.

◊ OESO / EOHW REVIEW: All protocols and amendments are reviewed by OESO and EOHW to assure compliance with Duke requirements for a safe workplace. You will receive an Email notice from OESO/EOHW concerning any issues that require attention. Please do not delay in responding to OESO/EOHW. Animal protocols may be reviewed by the IACUC, but the protocol approval will not be granted until PROTOCOL CLEARANCE has been received from OESO and EOHW.

◊ PRIMARY IACUC MEMBER REVIEWER: A member of the IACUC will be assigned as the PRIMARY REVIEWER. A few days before the IACUC meeting you may receive an Email from the PRIMARY REVIEWER requesting clarification of certain points. Please reply to the Email with the information requested. The PRIMARY REVIEWER will be your advocate at the IACUC meeting.

◊ NOTICE OF IACUC REVIEW: Within 3 business days of the IACUC meeting you will be advised of the outcome of the Committee’s review. If approved, you will be given a protocol registry number and instructions on annual reporting of your animal use activity. If additional clarifications are required to secure approval, you will receive a point by point breakdown of necessary actions to secure approval.

PROTOCOL MAINTENANCE:

⇒ ANNUAL PROTOCOL REPORT: A request for an annual report of your animal use activities will be sent via Email during the 10th month of the protocol. Please complete and return the annual report promptly, as IACUC approval is required by the 12 month anniversary, or the protocol may be suspended by the IACUC.

⇒ USDA REPORT: The Duke animal program must report animal use to the USDA annually. A request for your animal numbers used will be sent via Email each fall. Please reply as soon as possible.

⇒ AMENDMENTS TO APPROVED ACTIVITY: Any change of research direction, addition of new procedures or personnel, or changes in approved procedures must be IACUC approved prior to performing the changed activity. The Amendment form is available on the animal program website under ‘FORMS AND REPORTS.’ Depending upon the nature of the change, amendments may take between 3 and 14 business days for approval.

⇒ DE NOVO REVIEW: According to NIH/PHS Policy, all protocols have a life cycle of 3 years. Projects continuing beyond 3 years must have a new protocol approved by the IACUC by the 36th month, or must be terminated. At the 33rd month of your protocol life cycle, OAWA will alert you by Email to submit a new protocol.

⇒ POST APPROVAL MONITORING: Duke has an active program of Post Approval Monitoring (PAM). Compliance Liaisons will occasionally monitor animal procedures and confirm that the laboratory practices are as described in the approved protocol. Most laboratories will receive one visit per year, but depending upon the type of research, more monitoring sessions may be required. Researchers should view this process as partnering with the IACUC to assure program integrity. Deficiencies noted will be addressed quickly and in a collegial manner.
Helpful Hints to Prepare Your Laboratory for an IACUC Inspection

February begins the next cycle of laboratory and housing space inspections by the IACUC. To better prepare your laboratory for IACUC inspection, consider performing an internal laboratory audit using the following questions to help guide your preparation:

Animal Housing:
◊ Are the animals clean and dry?
◊ Do the animals have adequate space to freely move around?
◊ Are cages constructed to prevent animal injuries?
◊ Do the animals appear to be comfortable?
◊ Are animals safely confined?
◊ Are the facilities adequately ventilated such that no odors (e.g. ammonia) are present?
◊ Are animals separated according to health status?
◊ Are animals properly identified (by individual tags or with cage cards)?
◊ Outdoor housing: Do outdoor shelters protect animals from the elements?
◊ Outdoor housing: Are shelters free of waste and dry?
◊ Outdoor housing: Are animals acclimated to outdoor shelters prior to placement?
◊ Are cage sizes appropriate for the species? (See The Guide for specific size recommendations),

Feed/Water
◊ Are feeders/waters easily accessible by the animals:
◊ Are feeders/waters free of feces and urine?
◊ Are food supplies stored off the floors?
◊ Are food supplies sealed and marked?
◊ Are food supplies used within 180 days of milling?
◊ Are feeds free of vermin contamination?
◊ Are regular feeding schedules followed?
◊ Is water available ad libitum and are automatic watering devices working properly?

Bedding:
◊ Is the bedding species appropriate?
◊ Is bedding clean and dry?
◊ Is the bedding being stored off the floor on racks or skids?

Animal Social Environment:
◊ Are social animals group housed and non-compatible species housed separately?
◊ Have enrichment devices been supplied?
◊ Do animals appear content and without distress?

Physical Environment/Physical Plant:
◊ Are all surfaces sanitizable (Any fabric chairs? Adhesive tape on walls, etc.)?
◊ Are support facilities well maintained (food storage and cage washing areas clean)?
◊ Are the floors, work areas, and corridors clean and free of debris?
◊ Are floor drains clean and traps filled with water?
◊ Are noise levels at a minimum?
◊ Is there evidence of pint that is chipped or cracked?

Room Temperatures/Humidity:
◊ Are room temperatures stable (24 hour reading should not change more than 4 degrees)?
◊ Is the humidity stable (Humidity levels should be between 30 and 70%)?

Room Lighting/Air Flow:
◊ Is the lighting functioning properly?
◊ Are there objectionable odors in the animals spaces?
◊ Does the air smell stagnant or foul?
◊ Do rooms have proper directional air flow?

Sanitation:
◊ Are animal areas separate from personnel areas?
◊ Animal products and human food stored in separate refrigerators?
◊ Are primary enclosures sanitized regularly?
◊ Are wastes disposed of properly?
◊ Are there any signs of vermin infestation?

Record Keeping and Postings:
◊ Are all animals properly identified?
◊ Were animals observed and cared for daily?
◊ Are complete clinical records maintained for those animals that require them?
◊ Is the appropriate security/warning signage posted?
◊ Is the ‘Whistleblower Policy’ posted?
◊ Is the animal protocol available?
◊ Are appropriate monitoring procedures in place?
◊ Is the emergency contact list posted?
◊ Has the IACUC approved exceptions to The Guide?

Emergency, Weekend and Holiday Care:
◊ Are emergency contacts current and posted?
◊ Are there provisions for routine animal care during holidays and on weekends?
◊ Are the room sheets documented during holidays and on weekends?
◊ Do all lab members know how to contact DLAR veterinary staff after hours if needed?

Procedure Areas, Non-Survival Surgeries, Laboratories, Rodent Surgeries:
◊ Are sharps disposed of properly (needles are uncapped & in sharps containers)?
◊ Are cylinders of compressed gas immobilized (chained to walls)?
◊ Are carcasses disposed of properly? (Biohazard labeled freezer)
◊ Are procedures in place to monitor and scavenge anesthetic gases?
◊ Has the hood where animal procedures are performed been inspected within the past year?
◊ If an anesthetic vaporizer is used, has it been properly calibrated within the appropriate time frame?
Mouse Breeding 101:
How to optimize your colony management

Many laboratories on campus breed mice as part of their animal work. One common problem encountered while maintaining or breeding a specific colony includes being able to maintain/expand your colony within your allocated space without creating overcrowded cages. It is important that we partner to help to avoid this concern as well as optimize breeding to produce appropriate numbers of experimental animals within the desired time frame for research.

Some of the topics that will be covered include: the pros and cons to various breeding paradigms, animal weanings/transfers, and how to avoid creating overcrowded cages and maintain compliance with Duke’s Policy on “Cage Density Requirements for Mice.” We hope to have DLAR representation at this meeting as well to assist in any facility issues that laboratories may be encountering and to supplement recommendations for breeding given individual situations and needs.

The presentation will be on February 18th, 2007 in room 103 of the Bryan Research Building, located at 421 Research Drive, on Duke University's West Campus.

Attendees are encouraged to bring a lunch. OAWA will provide drinks and desserts. The session will begin promptly at noon. Please arrive early to sign-in and find a seat.

For those who will be coming from off campus, driving directions and parking information can be found at the following link: http://neuro.duke.edu/Links/map.htm

This session will count for 1 CEU of AALAS In-house Training Credit