Animal Care Matters

An IACUC and ARF e-Newsletter

Summer & Fall 2010

From the IACUC Administrator — Stephanie Cook

- CHANGES TO THE AUP REVIEW PROCESS -

Several changes were recently made to the IACUC's review process. Some were made to come into compliance with current regulations, and others to facilitate the approval process for investigators. Please read through the description of the changes.

I. CHANGES TO THE REVIEW PROCESS: A new review process was passed at the September 2010 IACUC meeting. This process will eliminate automatic full committee review for many new Animal Use Proposals, allowing certain proposals to be forwarded to Designated Member Review (DMR) without FCR. This will shorten the time required for meetings and approvals; it is estimated that approximately 50% of new proposals will be routed directly to DMR.
The following description is taken from information to be included in the 2010 OLAW Annual Report and amended PHS Assurance document:

> Requests for animal use are made by submitting an Animal Use Proposal (AUP) form to the IACUC. A complete copy of the AUP is sent to each member of the IACUC. AUPs may either undergo Designated Member Review (DMR) or Full Committee Review (FCR). Protocols that involve (1) survival surgery, (2) activities that fall under USDA Pain and Distress Category E, (3) protocols involving USDA covered species, or (4) other protocols as determined by the IACUC chair undergo FCR. All other AUPs are eligible for DMR.
>
> Designated Member Review
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> *All* IACUC members are provided with a copy of the AUP and allowed a reasonable amount of time to request FCR. If no member requests FCR, then the Animal Use Protocol undergoes DMR.
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> For DMR, the protocol is reviewed by the IACUC Chair, the Attending Veterinarian and one or more Designated Reviewer(s) who are assigned by the IACUC Chair. After reviewing the AUP, the reviewers may (1) approve the AUP, (2) require modifications to secure approval or (3), require FCR. If there is not a unanimous decision among the IACUC Chair, the Attending Veterinarian and the Designated Reviewer(s), then the AUP is referred to the IACUC for FCR. AUPs approved by DMR are included in the minutes of the following IACUC meeting.
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> Full Committee Review
>
> Two committee members are assigned as principal reviewers for each AUP. The principal reviewers present their analysis of the AUP to the committee at a convened meeting. All members are afforded the opportunity to comment and/or ask questions about the AUP. Members who cannot attend a meeting may relay questions or comments via

INSIDE THIS ISSUE:

SEND YOUR ORIGINAL ANIMAL ORDER FORMS TO ARF AFTER YOU HAVE FAXED THEM

ANIMAL ORDER DEADLINE

Thursdays @ 2:00pm is the cutoff for ordering animals needed the following week. This allows the ARF sufficient time to clarify any issues that may arise due to incomplete information from the PI as well as unforeseen vendor problems. If you are ordering animals, please make someone familiar with the order available on Thursdays to answer questions. Theresa wants you to have your animals when you need them.

From the IA: GOODBYE, HELLO, AND STAY TUNED -

GOODBYE TO Dr. Ken Walsh, Associate Professor in the School of Medicine's Department of Pharmacology, Physiology and Neuroscience, who stepped down as the IACUC Chair in June. At our 2010 committee retreat, Dr. Walsh was recognized by the Office of Research Compliance for his 16 years of service to the University and 6 years as Chair.

Although Dr. Walsh's expertise will be missed, the committee is happy to see Dr. Edie Goldsmith take the reins of leadership. Dr. Goldsmith is an Associate Professor in the School of Medicine's department of Cell Biology and Anatomy. She has served on the IACUC as an alternate member, a full member, and as Vice-chair of the committee. HELLO to Dr. Goldsmith!

STAY TUNED — Important changes are taking place in IACUC administration. The committee recently changed its review process from one in which nearly all new business was reviewed by full committee, to a Designated Member Review (DMR) process. It is anticipated that this process will be facilitate approvals by reducing or eliminating time delays.

New policies on decapitation and environmental enrichment have been adopted—please read!.

The new ARF/IACUC/IBC software is in the early development stages and is planned for roll-out early in 2011.

- Stephanie Cook

AUP REVIEW CHANGES (cont'd)

the Chairperson, another committee member, or the IACUC Administrator. A quorum must be present at the meeting to conduct reviews and vote. Following the committee review, the members may vote to (1) approve, (2) require modifications, or (3) withhold approval of the AUP. If by majority vote the IACUC requires modifications of an AUP to secure approval, the committee will (1) assign one or more members as designated reviewers for the modified AUP or (2) require FCR of the modified AUP. The default process is for the modified AUP to be reviewed by a designated reviewer; however, any committee member, present or not, may require FCR of the modified AUP. If the IACUC uses a Designated Reviewer for the modified AUP, the IACUC Chair assigns one or more members who may (1) approve the modified AUP, (2) require additional modifications to secure approval, or (3) refer the AUP back to the IACUC for FCR. If more than one Designated Reviewer is assigned, the modified AUP must be approved unanimously by the Designated Reviewers or it must undergo FCR. If the IACUC votes to withhold approval the investigator may present a new AUP for *de novo* review.

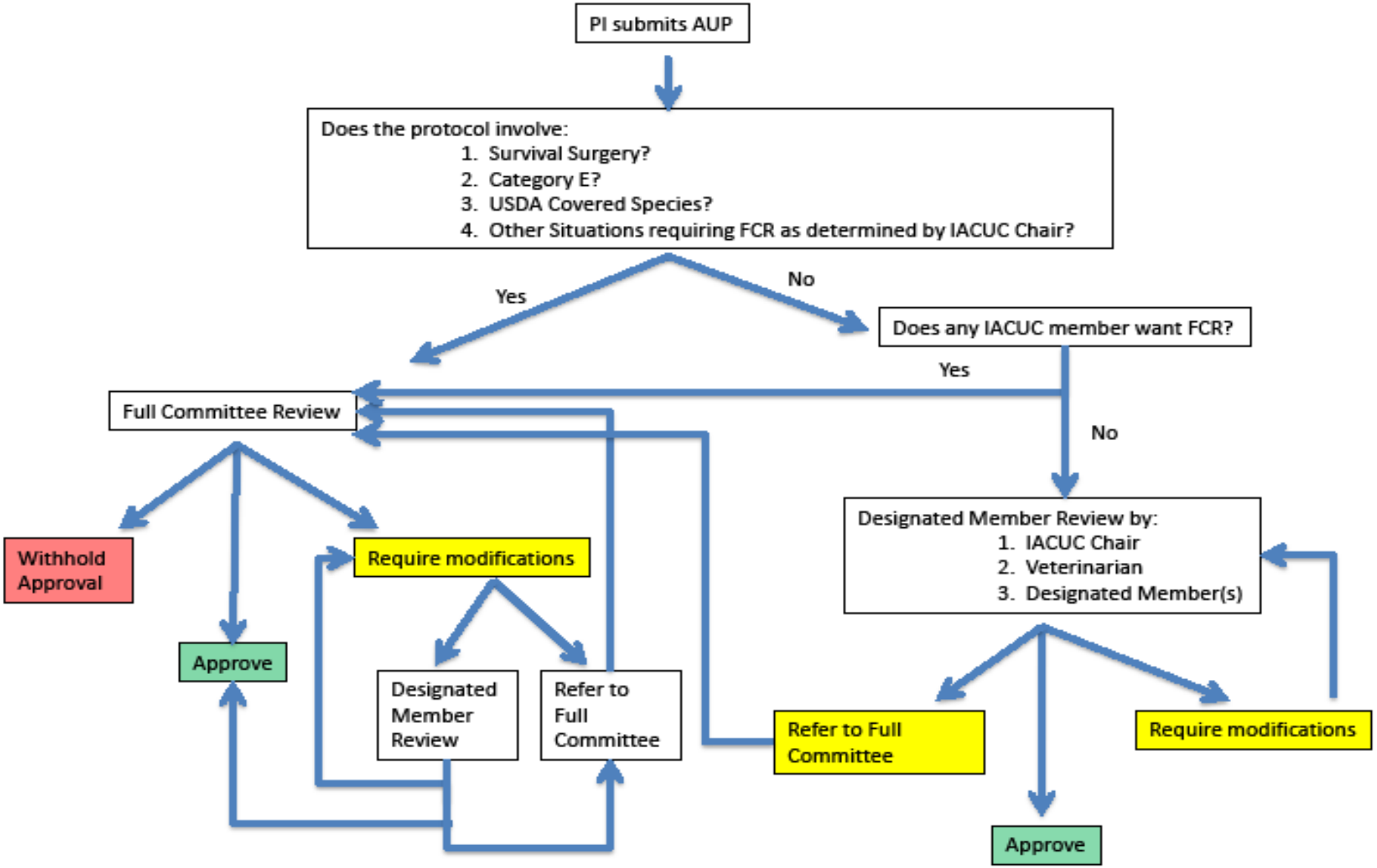
II. WHAT THE CHANGES MEAN TO INVESTIGATORS

A. If your AUP meets any of the following conditions, your AUP will automatically go to FCR (Full Committee Review); otherwise, it will be assigned to DMR (Designated Member Review). All non-administrative amendments go to FCR.
   1. Use of survival surgery
   2. Animals requiring placement in Category E
   3. Use of USDA-covered species
   4. Any other situation/condition determined to require FCR, as determinted by the IACUC chair

   PLEASE NOTE: DMR DETERMINATIONS ARE MADE WITHIN 2 DAYS OF RECEIPT. IF YOU ANTICIPATE DMR ASSIGNMENT, PLEASE SUBMIT YOUR APPLICATION AS SOON AS POSSIBLE SO ASSIGNMENT AND REVIEW CAN BEGIN. THERE IS NO NEED TO WAIT FOR THE PUBLISHED APPLICATION DEADLINE..

B. What happens after FCR? You will receive a letter based on the decision of the committee:
   1. Withhold approval: The AUP must be re-submitted as a new AUP (addressing the points found in the review) for FCR.
   2. Modifications requested to secure approval—you must submit a revision and cover letter addressing the modifications requested in the letter.
   3. Approved: The proposal or amendment is approved.

The New IACUC Review Process
At a Glance



IACUC POLICY #29
EUTHANASIA BY CERVICAL DISLOCATION OR DECAPITATION

BACKGROUND

The IACUC is specifically charged with reviewing the methods of euthanasia for each research protocol to assure compliance with the recommendations contained in the AVMA Guidelines on Euthanasia. Since physical methods of euthanasia require the most skill to perform and are most likely to be affected by human error, the AVMA recommends that such methods are used only when alternative methods are not appropriate. The USC IACUC requires anesthesia prior to decapitation or cervical dislocation unless there is an approved justification in the IACUC protocol. Physical euthanasia without anesthesia will be considered by the IACUC on a case by case basis.

TRAINING REQUIREMENTS

The IACUC reviews all protocols using physical techniques to assure that personnel performing the procedures are appropriately trained. The primary responsibility for establishing and monitoring this training lies with the investigator. Before using physical methods, inexperienced persons should be trained by experienced persons by an IACUC-approved trainer and should practice on carcasses or anesthetized animals to be euthanized until they are proficient in performing the method properly and humanely:

1) The trainer will demonstrate the decapitation procedure to one or more researchers, the attending veterinarian or his designee.
2) The researcher(s) will (each) practice the procedure on anesthetized or dead rodents until proficient. The trainer will be present for each of these practice decapitations.
3) The researcher will then perform a live decapitation under the supervision of the trainer. This will be repeated (including additional anesthetized/dead decapitations, at the discretion of the trainer) until the researcher demonstrates proficiency.
4) Proficiency will be determined by the trainer, and will be based upon one or more demonstrations that the researcher conducts the decapitation quickly and smoothly, without any overt signs of distress in the animal.
5) If animals are required for training, the Principal Investigator will request those animals on the relevant protocol or consult with Animal Resource Facilities about using animals from the Institutional Training Protocol.
6) Upon completion of training / demonstration of proficiency, the trainer will document the proficiency and provide it in writing to the IACUC. A copy of the document will be kept in the IACUC office.
7) Researchers who are approved to perform live decapitations must be listed on the appropriate IACUC protocol.

ACCEPTABLE USE

Use of cervical dislocation to euthanize mice and rats with body weights <200g by trained personnel is appropriate (upon IACUC approval) if the investigator has considered other methods, and has determined that cervical dislocation without the use of other agents is the most appropriate method based the specific aims of the study. The USC IACUC requires anesthesia prior to cervical dislocation unless there is an approved justification in the IACUC protocol.

Decapitation can be used to euthanize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated when performed without anesthesia. It also provides a means of obtaining anatomically undamaged brain tissue for study. Handling and restraint required to perform this technique may be distressful to animals. Distress is largely minimized in animals that are handled regularly and are accustomed to being picked up by the investigators.

Decapitation may be aesthetically displeasing to personnel performing or observing the technique. Guillotines that are designed to accomplish decapitation in adult rodents and small rabbits in a uniformly instantaneous manner are commercially available. ***(continued on pg. 5)***

CD and Decapitation Policy (cont'd)

Guillotines are not commercially available for neonatal rodents, but sharp scissors can be used for this purpose. The USC IACUC requires anesthesia prior to decapitation unless there is an approved scientific justification in the IACUC protocol, such as published reports or documentation of interference of the results by anesthetics.

METHOD FOR CERVICAL DISLOCATION

Before using the technique of cervical dislocation it should be practiced on deeply anesthetized rodents until the operator is competent.

Restrain the rodent in a normal standing position on a firm, flat surface and grasp the base of the tail with one hand.

Place a stick-type pen, a rod-shaped piece of sealed wood or metal, or the thumb and first finger of the other hand against the back of the neck at the base of the skull.
To produce the dislocation, quickly push forward and down with the hand or object restraining the head while pulling backward at a 30 degree angle from the table with the hand holding the tail.

Performing the procedure on a surface that the animal can grip may make it easier to gain access to the base of the skull because rodents often stretch themselves forward when held by the tail. The effectiveness of dislocation can be verified by separation of cervical tissues. When the spinal cord is severed, a 2-4 mm space will be palpable between the occipital condyles and the first cervical vertebra. Occasionally, however, the dislocation occurs between thoracic vertebrae. Check closely to confirm respiratory arrest, and when possible verify, by palpation, that there is no heartbeat.

METHOD FOR DECAPITATION

Those responsible for the use of this technique must ensure that personnel who perform decapitation techniques have been properly trained to do so. Personnel performing this technique should recognize the inherent danger of the guillotine and take adequate precautions to prevent personal injury. This technique is conditionally acceptable if performed correctly, and it should be used in research settings when its use is required by the experimental design and approved by the IACUC. The USC IACUC requires anesthesia prior to decapitation unless there is an approved justification in the IACUC protocol. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades and proper alignment and contact between blades (this can be checked by cutting a piece of folded paper). Rodents acclimated to being handled are calmer, less stressed, and facilitate the process.

GUIDELINES FOR THE DECAPITATION PROCEDURE:

The rodent will be removed from its home cage or experimental environment, anesthetized (if applicable) and carried to the guillotine.

A minimal number of animals should be brought into the decapitation room at a time while decapitations are being conducted. Ideally, each animal should be brought into the room individually.

The amount of time the animals are in the decapitation room with the guillotine should be kept to a minimum to prevent stress.

Every effort should be made to make sure the animal is not agitated prior to placing the animal in the guillotine.

The use of plastic cones (Decapicone® or other similar device) when using a guillotine, is optional but may reduce stress from handling, minimize the chance of injury to personnel, and improve the positioning of the animal in the guillotine.
(cont'd on page 6)

CD and Decapitation Policy (cont'd)

The researcher will hold the rodent securely, and place the rodent on the stage at the entrance to the guillotine and place the rodents head through the guillotine opening.
Once the head is in position, rapidly depress the guillotine lever.

One good technique is for the researcher to grasp the rodent gently but firmly around the back and push his/her hand slightly forward. This will push the front legs up, which prevents the rat from biting the investigator and also facilitates placing the animal in the guillotine.

MAINTENANCE OF GUILLOTINES/DECAPITATORS:

It is not recommended that guillotines be moved from room to room. If it must be moved to a different animal facility room or lab, sanitize the guillotine with disinfectant before moving to another location and disinfect again before placing it back into the original room.
After use on an individual animal, the guillotine must be rinsed and cleaned to remove blood, tissue and gross contamination. This is a critical step, not only because of contamination, but also because rodents will be stressed if they smell blood on the guillotine. A clean guillotine helps the rodents to remain calm, thereby making the procedure much easier and safer for the animal and the investigator.

Between decapitation sessions, and once gross contaminants have been removed, the entire unit should be thoroughly cleaned.
Rinse a final time with 70% alcohol to ensure evaporation and reduce the need to hand dry the equipment.

Turn the guillotine upside down with blades open to facilitate drying.

Ensure the guillotine is lubricated properly by applying silicon or 3-in-1 oil as necessary.
Guillotine blades must be kept sharp at all times. Frequency of sharpening may vary depending on the frequency of use and the species and number of animals decapitated. The IACUC recommends that blades must be sharpened every twelve months. Any reputable company may provide blade sharpening at a minimal cost.

It is recommended to have a spare guillotine available due to potential turnaround times for blade sharpening.

SAFETY CONCERNS FOR PERSONNEL:

1. Always make sure hands and fingers are clear of the blade path.
2. Only trained personnel should sharpen blades, lubricate the guillotine or take it apart.
3. Do not use decapitation equipment unless properly trained.
4. Old guillotine blades should be discarded in the sharps container.
5. Documentation: Maintain a log book of the date of blade sharpening or replacement in close proximity to the equipment.

PLEASE NOTE THAT A 'TRAINER' IS A PI OR OTHER STAFF APPROVED TO PERFORM THESE PROCEDURES AS OF SEPTEMBER 2010).

ARF Personnel Changes

Teresa Gibson, LATg is the Animal Resource Facility's new Quality Control coordinator. In addition, she now handles all the animal orders (they are still due by 2:00pm on THURSDAYS). She takes the place of Charlotte Joyner, who retired in July after 32 years. Teresa has spent many years as the SoM facility supervisor.

Jennifer Woodruff, AS—Veterinary Technology, is now the acting SoM facility supervisor.

IACUC POLICY #30
ENVIRONMENTAL ENRICHMENT FOR ALL SPECIES

BACKGROUND

This policy outlines the types of standard housing used for laboratory animal species at USC and the types of environmental enrichment materials or practices that may be used to enhance species-specific behavior and reduce distress and anxiety in laboratory animals.

The *Guide for the Care and Use of Laboratory Animals*[1] ("*The Guide*") states that:

> *"A good management program provides the environment, housing, and care that permit animals to grow, mature, reproduce, and maintain good health; provides for their well-being; and minimizes variations that can affect research results."*
>
> *"Animals should be housed with the goal of maximizing species-specific behaviors and minimizing stress -induced behaviors."*
>
> *"Depending on the animal species and use, the structural environment should include resting boards, shelves or perches, toys, foraging devices, nesting materials, tunnels, swings, or other objects that increase opportunities for the expression of species-typical postures and activities and enhance the animals' well-being."*
>
> *"Consideration should be given to an animal's social needs. The social environment usually involves physical contact and communication among members of the same species (conspecifics), although it can include non-contact communication among individuals through visual, auditory, and olfactory signals."*

The Animal Welfare Act (AWA) mandates "environmental enrichment" for nonhuman primates[2] by specifying that there must be a "program for the psychological well-being of nonhuman primates". Exercise requirements for dogs[3] are also specified in the AWA.

Definitions

Standard housing refers to the type(s) of housing approved by IACUC and ARF for housing the species concerned.

Environmental enrichment refers to additions to an animal's environment with which it can interact. The goal is to allow animals to express a range of species-typical behaviors which may enhance their well-being. Examples of environmental enrichment include the following:

- Allowing control over the environment such as providing opportunities for:
  - nest-building
  - areas for animals to hide from threatening situations
  - exercise
- Novel items (e.g. food, toys, climbing structures).
- Group housing to allow interaction with conspecifics.

The type of environmental enrichment provided depends on the species of animal, type of housing, space available, research needs, husbandry practices and other operational needs.

POLICY STATEMENT

In compliance with Federal Animal Welfare Regulations and guidance and in consideration of the physical and social needs of research animals the IACUC requires that appropriate environmental enrichment be provided to standard animal

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Animal Care Matters is published four times a year by the Institutional Animal Care and Use Committee (IACUC) and Animal Resource Facilities (ARF) of the University of South Carolina (USC).

The IACUC is an institutional body appointed by the USC President to oversee the program for the humane care and use of all vertebrate animals used for research, teaching, and training. Any investigator who intends to use laboratory animals must submit an Animal Use Proposal (AUP) to the IACUC for its review and approval.

The ARF provides care and maintenance of all animals used by investigators. Preventive care is provided through vendor animal health evaluations, quarantine programs, and sentinel animal diagnostics. Special care and services can be provided upon request.

Comments and submissions for **Animal Care Matters** are welcome and should be directed to Stephanie Cook, IACUC Administrator, at 777-8106 or *iacuc@mailbox.sc.edu*.

IACUC Meetings 2010

Meeting Date	AUP Deadline
Jan 7	Dec 16
Feb 4*	Jan 28
Mar 4	Feb 24
Apr 1	Mar 24
May 6	Apr 28
Jun 3	May 26
Jul 1	Jun 23
Aug 12*	Jul 28
Sep 2	Aug 25
Oct 7	Sep 29
Nov 4	Oct 27
Dec 2	Nov 17

*semi-annual

IACUC MEMBERSHIP

CHAIRMAN
Edie Goldsmith, Ph.D.

MEMBERS
Shayne Barlow, D.V.M., Ph.D.
Greg Brower, D.V.M., Ph.D.
Raja Fayad, Ph.D.
Robert Lawrence, Ph.D.
Jennifer McLarty
Marj Peña, Ph.D.
Lawrence Reagan, Ph.D.
Theresa Smith, Ph.D.
Gabor Szalai, Ph.D.
Dave Volz, Ph.D.

COMMUNITY MEMBERS
Tina Forsthoefel, Ph.D.
Marli Clary Drum

CONSULTANT
Tommy Coggins

POLICY #30: ENVIRONMENTAL ENRICHMENT FOR ALL SPECIES
TABLE OF STANDARD AND ADDITIONAL ENRICHMENT

Species (common name)	Standard Housing	Standard Environmental Enrichment Required (ARF-Provided)	Additional Enrichment Allowed/Recommended (Research Group Must Provide)
Mice	Solid-bottom plastic cage with a wire bar lid that serves as a food hopper and water bottle holder. Filter top (microisolator lid). Cages may be placed on ventilated racks providing filtered air directly to the cage, or placed on static racks. Water bottle. Contact bedding consisting of commercially-available corn cob particles, wood chips, cotton, or paper products specifically made for laboratory animals. Commercially-available laboratory rodent diets approved by ARF.	Group-housed as appropriate (e.g. adult males from different litters cannot be housed together). Nesting material made from paper or cotton fibers. (e.g. Nestlets).	Disposable cardboard mouse houses ("Shepherd Shacks") Plastic mouse houses (must be cage-washer safe).
Peromyscus spp.	Solid-bottom opaque plastic cage with a wire bar lid that serves as a food hopper and water bottle holder. Cages are placed on static racks. Water bottle. Contact bedding consisting of commercially-available corn cob particles, wood chips, cotton, or paper products specifically made for laboratory animals. Commercially-available laboratory rodent diets approved by ARF. Opaque housing.	Group-housed as appropriate Nesting material made from paper or cotton fibers. (e.g. Nestlets).	Disposable cardboard mouse houses ("Shepherd Shacks") Plastic mouse houses (must be cage-washer safe).
Rats	Solid-bottom plastic cage with a wire bar lid that serves as a food hopper and water bottle holder. Filter top (microisolator lid) Cages may be placed on ventilated racks providing filtered air directly to the cage, or placed on static racks. Water bottle. Contact bedding consisting of commercially-available corn cob particles, wood chips, cotton, or paper products specifically made for laboratory animals. Commercially-available laboratory rodent diets approved by ARF.	Group-housed as appropriate (e.g. adult males are often incompatible). Sterilized Nylabones for chewing	Sterilized wooden tongue depressors for chewing. Plastic rat houses (must be cage-washer safe). Plastic drain pipes or connectors

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Rabbits	Sanitizable cage of appropriate size with suspended floor. Water bottle or lixit for automatic watering. Commercially-available laboratory rabbit chow approved by ARF.	Sanitizable toys such as plastic chains or balls, stainless steel rings, PVC pipe. Small portions of timothy hay, other grass hay, alfalfa hay, fresh spinach or fresh kale offered one to three times per week in a sanitizable feeder, or on cage bottom.	Group-housed as appropriate. Small amounts of other fresh vegetables such as carrots and Romaine lettuce. Small amounts of dried banana chips apples or pineapple.
Pigs	Indoor pens with sanitizable surfaces. Lixit for automatic watering. Wood chip contact bedding in solid bottom pens. Sanitizable food bowls. Nutritionally complete commercially available diet for laboratory pigs approved by ARF.	Group-housed if pen size is adequate and if animals arrive together and are compatible. Social contact with other pigs if possible. Positive human interaction (e.g. patting, scratching, rubbing back), if pigs are acclimatized to this. Sanitizable toys (e.g. Kong toys, plastic balls).	Small amounts of food treats such as fresh vegetables, yogurt or fruit.
Cats	Housed in large indoor holding rooms in compatible groups. Space allocation is at least the minimum required by Animal Welfare Act. Cats may be housed in smaller individual cages temporarily (e.g. after surgery). Sufficient litter boxes appropriate for the number of cats. Fresh water in sanitizable bowls. Nutritionally complete, commercially available dry cat food approved by ARF. • Group-housed as appropriate.	Group-housed as appropriate. Positive human interaction daily (e.g. patting, scratching, rubbing back). Multi-level shelves for resting and climbing. Sanitizable or washable toys and items such as scratching posts, toy mice and balls. Guinea Pigs	

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Species (common name)	Standard Housing	Standard Environmental Enrichment Required (ARF-Provided)	Additional Enrichment Allowed/Recommended (Research Group Must Provide)
Guinea Pigs	Polystyrene, polycarbonate or other high-temp plastic solid bottom cage with a wire bar lid that serves as a water bottle holder. Filter top (microisolator lid) may be used. Cages may be placed on ventilated racks providing filtered air directly to the cage, or placed on static racks. Water bottle. Sanitizable food bowl. Contact bedding consisting of commercially-available corn cob particles, wood chips, cotton, or paper products specifically made for laboratory animals. Commercially-available laboratory guinea pig diets approved by ARF.	Group-housed as appropriate.	Plastic guinea pig houses (must be cage-washer safe). Disposable cardboard guinea pig houses ("Shepherd Shacks"). Small quantities of Timothy or alfalfa hay. Sanitizable toys such as balls or Kong toys.
Hamsters	Solid-bottom plastic cage with a wire bar lid that serves as a food hopper and water bottle holder. Filter top (microisolator lid). Cages may be placed on ventilated racks providing filtered air directly to the cage, or placed on static racks. Water bottle. Contact bedding consisting of commercially-available corn cob particles, wood chips, cotton, or paper products specifically made for laboratory animals. Commercially-available laboratory rodent diets approved by ARF.	Group-housed as appropriate. Nesting material made from paper or cotton fibers.	Disposable cardboard hamster houses ("Shepherd Shacks") Plastic hamster houses (must be cage-washer safe).
Gerbils	Solid-bottom plastic cage with a wire bar lid that serves as a food hopper and water bottle holder. Filter top (microisolator lid). Cages may be placed on ventilated racks providing filtered air directly to the cage, or placed on static racks. Water bottle. Contact bedding consisting of commercially-available corn cob particles, wood chips, cotton, or paper products specifically made for laboratory animals. Commercially-available laboratory rodent diets approved by ARF.	Group-housed as appropriate. Nesting material made from paper or cotton fibers.	Disposable cardboard gerbil houses ("Shepherd Shacks") Plastic gerbil houses (must be cage-washer safe).

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Species (common name)	Standard Housing	Standard Environmental Enrichment Required (ARF-Provided)	Additional Enrichment Allowed/Recommended (Research Group Must Provide)
Birds	Sanitizable wire cages or large flight cages. Perches. Fresh water in water bottle or sanitizable bowls. Food containers and water bottles should be designed and positioned to minimize fecal contamination. Shelter from sun, rain and extreme weather conditions if housed in outdoor aviaries. Nutirionally-complete food appropriate for the species. Dietary supplements appropriate for the species (e.g. cuttlefish bones, shell grit, fresh greens).	Group-housed as appropriate. Mirrors if housed singly. Nesting materials and enclosures for breeding.	Sanitizable mirrors, swings, ladders, or other types of perches as appropriate for the species.
Xenopus	Aquaria made of sanitizable materials. Static or flow-though water system. Water filtered or conditioned to remove/inactivate chlorine and chloramine. Nutritionally-complete commercially-available food.	Group-housed as appropriate. Shelter structures such as plastic guinea pig houses or large PVC pipes to allow frogs to hide.	Small amounts of dietary supplements such as blood worms, chopped beef heart, and chopped liver.
Fish	Aquaria made of sanitizable materials. Static or flow-though water system. Water filtered or conditioned to remove/inactivate chlorine and chloramine. Nutritionally-complete fish food appropriate for the species.	Group housed as appropriate.	
Reptiles	Sanitizable, secure enclosures of appropriate size and configuration for the species. Opportunity for animals to thermoregulate, or a constant temperature range in the thermoneutral zone for the species. Water and nutritionally complete food appropriate for the species.	Sanitizable objects to provide hiding places.	