

Handbook of Policies

Animal Care and Use Committee of Wake Forest University

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1. Animal Research

Animal Care and Use Committee of Wake Forest University

A. INTRODUCTION

The Animal Welfare Act (as amended in 1985) and NIH policy require an institutional committee to oversee the animal care and use program. This committee must be composed of at least five members and include an individual unaffiliated with the institution, a veterinarian with program responsibilities, a practicing scientist experienced in research involving animals and a nonscientist. The Animal Care and Use Committee (ACUC) is the institutional committee for animal care and use issues at Wake Forest University (WFU). The ACUC has University-wide oversight responsibilities regarding adequacy of facilities, animal husbandry, health care and appropriate use of animals in educational programs and research. It is an important element of WFU's compliance with Federal and state regulations, and plays an essential role in advising WFU administration, the Animal Resources Program (ARP) and research faculty and staff on matters related to the care and use of animals.

The President of WFU or his designee appoints members for rotating three-year terms. Members of the University ACUC include practicing scientists, nonscientists, veterinarians and at least one lay person not affiliated with the University.

B. PROTOCOL REVIEW

In keeping with the regulations set forth in The Public Health Service Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions (1985, revised 1986) and the Animal Welfare Act, WFU policy requires **all proposed activities using live vertebrate animals** to be reviewed and approved by the ACUC.

1. Review Process

Investigators must complete a standard ACUC protocol application form, which can be obtained from the Office of Research (716-4548). The review process is as follows:

a. Applications are submitted to the Office of Research, Wake Forest University School of Medicine, in accordance with published monthly deadlines. The Office of Research refers submissions to the ACUC for review.

b. Applications may be considered for expedited review by a designated reviewer(s), provided that the proposed studies do not involve the use of non-routine or harmful invasive procedures, prolonged restraint, the use of animals that have a serious natural or experimental disease that will be maintained in that state for an extended period of time, or methods of euthanasia that differ from those recommended by the AVMA. The review will be conducted by at least one member of the ACUC, designated by the Chair and qualified to conduct the review. The reviewer(s) shall be given the authority to approve, require modification (to secure approval) or request full committee review of the proposal. In addition, the protocol will be distributed to each committee member. Each member will critique the protocol and submit his/her recommendations to the ACUC secretary, within four working days, to be forwarded to the reviewer(s). Any member may require that the proposal be brought before a full, convened meeting. If a majority of the committee fails to respond, the protocol will be considered at the next full, convened meeting.

- c. All other applications are considered by the full committee at a convened meeting.
- d. The Committee convenes monthly to consider pending applications, review expedited actions taken and review progress of previously approved protocols if renewal beyond the initial one-year period is desired. Amendments or changes to previously approved protocols also are considered.
- e. The Committee votes on each application and a majority of members is needed for approval. One veterinarian and one member from a scientific discipline are necessary to achieve a quorum, which is defined as a majority of Committee members.
- f. No member may participate in the ACUC review or approval of an application or proposal in which the member may have a conflict of interest (e.g., is personally involved in the project); nor may a member who may have a conflict of interest contribute to the constitution of a quorum.
- g. As an alternative to convened meetings, each ACUC member may be provided with a list of applications and proposals to be reviewed. Those sections of applications and proposals that relate to the care and use of animals shall be available to all ACUC members, and any member of the ACUC may upon request, obtain full committee review of those sections. If full committee review is not requested, at least one member of the ACUC, designated by the Chair, and qualified to conduct the review, shall review those sections. The designated reviewer(s) shall have the authority to approve, require modifications (to secure approval), or request full committee review of those sections. If full committee review is requested, approval of those sections may be granted only after review at a convened meeting of a quorum of the ACUC and with the approval vote of a majority of the quorum present. Historically, the alternative to convened meetings has been used very rarely, and it is intended for use only in unusual circumstances.
- h. The ACUC may invite consultants to assist in the review of complex issues. Consultants may not approve or withhold approval of an application or proposal or vote with the ACUC.
- i. The ACUC notifies investigators in writing of its decision to approve or withhold approval of those sections of applications or proposals related to the care and use of animals or of modifications required to secure ACUC approval. If the ACUC decides to withhold approval of an application or proposal, it must include in its written notification the reasons for its decision, and give the investigator an opportunity to respond in person or in writing. Applications are approved for three years.
- j. The ACUC conducts an annual continuing review of applications and proposals covered by this policy in the second and third years of the research proposal. Projects extending beyond this period must be resubmitted to the ACUC as a full protocol rather than a renewal request.
- k. Applications and proposals that have been approved by the ACUC may be subject to further appropriate review and approval by officials of the institution. However,

those officials may not approve those sections of an application or proposal related to the care and use of animals if they have not been approved by the ACUC.

2. Activities Requiring Full Committee Review

a. Use of Non-routine or Harmful Invasive Procedures

It is the policy of the WFU ACUC that protocols which involve non-routine or harmful invasive procedures such as major multiple survival surgery are not eligible for expedited review, and must always be reviewed by the full Committee. In most instances, multiple survival surgical procedures on a single animal are discouraged. However, under special circumstances, more than one major surgical procedure on a single animal may be permitted provided they are related components of a research or instructional project. Major surgery is defined as any surgery in which a major body cavity is opened or produces substantial impairment of physical or physiological functions.

b. Prolonged Restraint

The WFU ACUC policy states that protocols which involve prolonged restraint are not eligible for expedited review and must always be reviewed by the full Committee. Standard tethering systems for rats and monkeys that allow full mobility within cage space are not considered prolonged restraint by the ACUC. This decision is based on years of experience with these techniques in several laboratories within the institution.

c. Use of Animals With a Serious Natural or Experimental Disease Maintained for an Extended Time

It is the policy of the WFU ACUC that the full Committee must always review protocols that involve the long-term maintenance of animals with serious debilitating diseases. An example of such a protocol is one including maintenance of animals with naturally occurring or experimentally induced paralysis.

d. Methods of Euthanasia Other Than Those Approved by the AVMA

The ACUC currently approves all methods of euthanasia that are in agreement with the 2000 Report of the AVMA Panel on Euthanasia recommendations. Exceptions to these recommendations are reviewed individually by the ACUC and approved when deemed acceptable. This requires scientific justification by the investigator and may require observation of the proposed method by ACUC members. (See ACUC policy on decapitation and cervical dislocation.)

3. Special Considerations

a. Lay Language

Federal law requires that certain portions of ACUC protocols be written in terms that a lay person can understand. The ACUC requests that investigators pay special attention to this requirement. Protocols that are not written in lay language, as determined by the ACUC lay representative(s), will be returned to the investigator for rewriting.

b. Anesthesia and Analgesia

Appropriate anesthesia and analgesia are essential in animal experiments involving painful or invasive procedures. The ACUC recommends that investigators consult with the Animal Resources Program veterinary staff on matters pertaining to

anesthesia and analgesia in animals before submitting a protocol for ACUC approval. Prior consideration often eliminates the need for protocol revision and thus expedites the review process.

c. Experimentation Involving Hazardous Agents

All animal studies involving the use of hazardous agents (living microorganisms, toxic chemicals and carcinogens or radioisotopes) must be approved by the appropriate committee(s) (i.e., Biosafety, Chemical or Radiation Safety) before the ACUC can approve an animal protocol.

i. Experiments using select agents; potentially infectious or toxic biological materials must be approved by the Biosafety Committee before approval by the ACUC can be obtained and studies initiated. Applications for Biosafety Committee approval (Memorandum of Understanding) may be obtained from the Environmental Health and Safety website - <http://www.wfubmc.edu/ehs/>

ii. Experiments using hazardous chemicals (caustic or carcinogenic) must be approved by the Chemical Safety Committee before approval by the ACUC can be obtained and studies initiated. Applications for Chemical Safety Committee approval may be obtained from the Environmental Health and Safety website – <http://www.wfubmc.edu/ehs>

iii. Experiments Using Radioisotopes

Animal studies involving the use of radioisotopes must be approved by the Radiation Safety Committee before approval by the ACUC can be obtained and studies initiated. Applications for such studies may be obtained from Environmental Health and Safety website - <http://www.wfubmc.edu/ehs>

d. Satellite Animal Housing Facilities

The policy of the WFU ACUC states that animals must be housed within an ARP-operated animal facility. An exception to housing animals within the central animal facilities is made on the basis of scientific requirements of a particular research project, and can be made only by the ACUC. Requests for such an exception must be submitted in writing to the ACUC and must specifically address the scientific justification for not housing the animals in the central facilities as well as each of the other points listed in the Policy on Housing Laboratory Animals. Permission must be granted by the ACUC before animals are housed anywhere outside the central facilities. ARP personnel are available for consultation concerning the laws and regulations that pertain to satellite facilities as well as those that address the husbandry and veterinary care of animals housed within them.

e. Satellite Surgery Facilities

Federal laws and guidelines govern survival surgery in all species of animals. In order to ensure institutional compliance with these regulations, it is the policy of the ACUC that all survival surgery be performed only in ACUC-approved facilities. Investigators must apply to the ACUC for approval of a satellite surgical facility. ARP personnel are available for consultation concerning the laws and regulations that pertain to rodent and nonrodent survival surgical facilities.

C. ACUC POLICIES

The ACUC has adopted policies that address specific animal care and use issues. Investigators using animals in research and teaching activities are urged to review these policies before completing ACUC protocol forms.

D. ADDITIONAL ACUC RESPONSIBILITIES

1. Review the institution's program for humane care and use of animals
2. Inspect, at least semi-annually, all of the institution's animal facilities, including satellite facilities
3. Review concerns involving the care and use of animals at the institution
4. Make written recommendations to the Vice-President and Dean regarding the institution's animal program, facilities or personnel training
5. Review and approve, require modifications in (to secure approval), withhold approval or propose significant changes regarding the use of animals in ongoing activities.
6. The ACUC may suspend a previously approved activity if it determines that the activity is not being conducted in accordance with applicable provisions of the Animal Welfare Act, or the Guide. The ACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the ACUC and with the vote of a majority of the quorum. If the ACUC suspends an activity involving animals, the institutional official in consultation with the ACUC shall review the reasons for suspension, take appropriate corrective action and report that action with a full explanation to the Office Laboratory Animal Welfare (OLAW), other NIH offices or other agencies as required.

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2. Institutional ACUC Training Program

Animal Care and Use Committee of Wake Forest University

The Animal Welfare Act (AWA) and Public Health Service Policy require that institutions receiving federal funds have a program to certify that any person (faculty, staff, students) performing procedures in live animals (anesthetized or not) or who is responsible for the welfare of an animal **in a research setting**, e.g., monitoring animals at any time before or after an experimental procedure, be certified by our Institutional ACUC training program (the Program). Because the Program addresses important issues related to the proper conduct of our research, this policy also applies to all such persons, including graduate or medical students who may have only brief exposure to animals during training rotations.

Research personnel who do not perform procedures on animals or have responsibility for their welfare are not required to take the Program to participate in a research project. Such people might include chemists, statisticians, and histology or biochemistry technicians who process tissue, but do not work with live animals. This policy also recognizes that the principal investigator of a research program (e.g., center director, lab chief) may not necessarily be the principal investigator of an animal protocol conducted as part of that program's efforts. In a case like this, the principal investigator of the research protocol must meet all requirements for that duty and be ultimately responsible for the welfare of the live animals used in that project. Whereas training and certification are strongly recommended for all personnel who will perform procedures on animals, we recognize that this is not feasible in all situations. Two specific instances where certification is not required are:

1. Students or other trainees **in a teaching situation** where certified personnel are responsible for the preparation and welfare of the involved animals. In this situation the certified personnel are expected to supply direct supervision over untrained personnel with regard to the welfare of the animals. Situations like this commonly exist in undergraduate teaching labs and in the training of physicians in new surgical procedures.
2. Visiting scientists who work at the institution for relatively short periods of time. Although we prefer that these scientists become certified, certification may not be required if the scientist is working as part of a team with certified staff on an approved protocol. While visits of less than two weeks might reasonably be conducted without training, a visit lasting more than two weeks, with repeated animal procedures, would require training.

Therefore, for Wake Forest University to be in compliance with Federal regulations, the Animal Care and Use Committee have developed a Program for the care and use of laboratory animals.

In the non-human research laboratory, the principal investigator will be responsible for ensuring the qualifications of the research personnel. The Animal Resources Program Director will be responsible for ensuring the qualifications of the animal care technicians and staff at the Bowman Gray, Chestnut, Reynolda, and Friedberg Campuses.

The IACUC Training Program is designed to provide basic information about the humane use of animals in biomedical research and teaching and includes the following topics.

1. The importance of animals in Biomedical Research and Teaching.
2. Basic needs of the species involved - husbandry, care and importance of the environment.

3. Proper handling and care for the animals used in research laboratories and classroom.
4. Proper selection and use of analgesics and tranquilizers to relieve pain and distress.
5. Proper use of anesthetics; principles of survival surgery and postoperative care.
6. Methods of euthanasia.
7. The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress.
8. Methods whereby a perceived deficiency in animal care and treatment is reported, including deficiencies in animal care and treatment reported by any employee of the facility. No facility employee, committee member, or laboratory personnel shall be discriminated against or be subject to any reprisal for reporting violations of any regulation or standards under the AWA;
9. Utilization of services (e.g., National Agriculture Library, National Library of Medicine) available to provide information:
 - (i) on appropriate methods of animal care and use,
 - (ii) on alternatives to the use of live animals in research,
 - (iii) that could prevent unintended and unnecessary duplication of research involving animals,
 - (iv) regarding the intent and requirements of the AWA.
10. If more specific needs on special procedures are required, "hands on" participation will be provided.

Administration of the Program

1. All Wake Forest University personnel, including faculty, postdoctoral fellows, research associates, research technicians, medical students, graduate students, and other individuals who are involved in vertebrate animal care, treatment and/or use are required to participate in the Institutional Animal Care and Use Committee (IACUC) Training Program.
2. As part of the program, the Laboratory Animal Training Association (LATA) administers online training courses and provides the means to document compliance with federal regulations on training in the humane care and use of laboratory animals. Program participants are required to successfully complete LATA courses based on the type of animal contact. All participants are required to complete the Occupational Health and the Laboratory Animal Modules, which covers topics of federal law and regulation, ethics of animal experimentation, alternatives to live animal use, and the responsibilities of the ACUC. Also, the module on Occupational Health is mandatory. Participants may complete species-specific LATA training modules when working with rats, mice, hamsters, guinea pigs, rabbits, dog, cats and nonhuman primates. LATA training modules for the conduct of surgery and the administration of anesthesia and analgesia in rodents are also provided for participants who will engage in these activities.
3. The principal investigator is responsible for identifying further training needs and providing specialized training. As an important supplement, the Animal Resources Program offers additional training upon request, and the IACUC periodically holds seminars on relevant topics.

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3. Expediting Protocols

Animal Care and Use Committee of Wake Forest University

1. When an expedited review is requested by the principal investigator, the protocol will be distributed to each committee member, providing it does not involve the following:
 - Use of non-routine or harmful invasive procedures,
 - Prolonged restraint,
 - Use of animals that have a serious natural or experimental disease and will be maintained in that state for an extended period of time,
 - Methods of euthanasia that differ from those recommended by the AVMA.
2. Each member will determine if the protocol may be expedited. Each member will critique the protocol and submit his/her recommendations to the ACUC secretary, within four working days, to be forwarded to the reviewer(s). Any member may require that the proposal be brought before a full, convened meeting. If a majority of the committee fails to respond, the protocol will be considered at the next full, convened meeting
3. The expedited review will be conducted by at least one member of the ACUC, designated by the Chair and qualified to conduct the review. The reviewer(s) shall be given the authority to approve, require modification (to secure approval) or request full committee review of the proposal. In addition, the protocol will be distributed to each committee member.
4. Majority vote of the full committee will determine if the protocol is expedited. Lack of response will be counted as a "no" vote. This should not be used as a means of casting a "no" vote.
5. Any member of the ACUC can request that the protocol be brought before a convened meeting of the whole committee.
6. If expedited review is accomplished, the protocol will not be presented at the ACUC monthly meeting for review. The Committee will be advised that a protocol has been expedited.
7. If a majority of members do not respond regarding the expedited protocol, it will be considered at the next convened meeting.

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4. Freedom to Alter Previously Approved ACUC Protocols

Animal Care and Use Committee of Wake Forest University

The Animal Care and Use Committee (ACUC) considered conditions under which investigators and personnel in teaching laboratories might be free to change experimental protocols previously approved by the ACUC. A distinction was made between those manipulations performed on animals just prior to death and those procedures done to animals expected to survive. The same approach was used for identifying investigator latitude in both instances. First, a category of "non-significant" was identified, for which full committee approval is not required. A second category, "significant changes," was also identified. Here, the investigator submits a brief protocol addendum to the chairman of the ACUC. The final category was reserved for those alterations in experimental procedures that require the submission of a new protocol for approval. The ACUC believes these provisions provide reasonable flexibility within regulatory requirements.

I. Conditions of Terminal Surgery/Euthanasia

- A. Non-Significant Changes (formerly "minor"; ACUC Chairman approval required; The changes include such alterations as:
1. The harvesting of additional tissues,
 2. The taking of additional measurements,
 3. A longer duration (provided anesthesia is adequate),
 4. Additional surgical manipulations during the terminal procedure
 5. Increase ($\leq 10\%$) in the number of animals

The objective is to encourage and allow investigators to maximize the amount of information collected from an animal about to be terminated and to permit a minor increase in the number of animals.

- B. Significant Changes (formerly "moderate"; ACUC chairman may approve with full committee concurrence; requires submission of an ACUC amendment). The changes include:
1. An alteration in the anesthesia procedure,
 2. An alteration in the method of euthanasia,
 3. A minor species change (one rodent species to another [mice to rats], one non-human primate to another [patas monkeys to green monkeys]), or
 4. Increase ($> 10\%$ and $\leq 25\%$) in the number of animals.

The objective is to ensure that any changes in the euthanasia/anesthesia procedures are consistent with the AVMA guidelines and to permit a nominal increase in the number of animals. It is necessary to monitor the number and type of species used for all experiments.

- C. Highly Significant Changes, (formerly "major"; these constitute a new experiment, thus a new protocol approval is required):
1. A change in experiment (e.g., if the experiment was originally approved for kidney studies but the investigator plans brain surgeries instead),
 2. A major change in species (e.g., from rodent to carnivore, from non-human primate to rodent), or

3. Increase (> 25%) in the number of animals.

The objective is to ensure that only approved studies are actually done. It is further necessary to ensure that investigators are capable of manipulating the particular type of animal to be used.

II. Conditions of Survival

- A. Non-Significant Changes, (formerly “minor”; no ACUC Chairman approval required). These changes include:
 1. Alteration in schedule,
 2. Additional sampling, or additional minor surgery, provided that the additional procedures do not involve more distress than procedures already approved (e.g., the addition of an intravenous glucose tolerance test to a protocol which already involves repeated blood sampling; or, the addition of a venous catheter to a protocol which already involves arterial catheterization; the electrical stimulation of additional or different nerves when such stimulation has already been approved; the addition of blood sampling to a protocol that already involves surgery), or
 3. An increase ($\leq 10\%$) in the number of animals

The objective is to allow the investigator the freedom to make those minor alterations in protocol which are required in the course of experimentation, while at the same time, ensuring that animals will not be exposed to an undue amount of distress.

- B. Significant Changes, (formerly “moderate”; ACUC chairman may approve with full committee concurrence, requires submission of an ACUC amendment). These changes include:
 1. The addition of minor surgery to an experiment that already involves anesthesia (e.g., subcutaneous fat or muscle biopsy),
 2. Alterations in surgical approach (e.g., paramedian approach instead of ventral midline approach),
 3. Alteration in choice of analgesics, anesthetics, etc.,
 4. A minor change in species (from one rodent to another (rat to mouse), from one non-human primate to another (green monkey to patas monkey)),
 5. Increase $> 10\%$ and $\leq 25\%$ in the number of animals used, or
 6. A series of non-significant changes (see above),

The objective is to ensure that any alterations in surgical techniques or approaches are consistent with approved practice. Also, it is imperative that investigators not make a series of non-significant changes to a protocol which, taken together, constitute a significant change.

- C. Highly Significant Changes, (formerly “major”; these constitute a new experiment, thus a new protocol approval is required). These changes include:
 1. The performance of major surgical procedures more frequently than proposed (e.g., renal wedge biopsy),
 2. Addition of anesthesia and/or surgery (major or minor) when a protocol does not include these items,

3. A major scientific change (e.g., if an investigator is able to complete the approved studies on a smaller number of animals than proposed, the remaining animals may not be used for purposes not approved),
4. A major change in species (rodent to carnivore, non-human primate to rodent),
5. Increase in the number of animals used (> 25%),
6. Procedures which alter the survival of the animals (e.g., survival of animals originally scheduled to be euthanized; changes in survival surgery resulting in a more difficult post-surgical course),
7. Multiple significant changes to protocol (see above), or
8. An alteration in housing of nonhuman primates and dogs that is inconsistent with the Wake Forest University Plan for environmental enrichment and psychological well-being of nonhuman primates and exercise for dogs (e.g., social to single cage housing).

The objective is to ensure that animals are not exposed to repeated major survival surgery without prior approval. Another objective is that all invasive manipulations have prior approval. Further, investigators must justify the need for a particular species as well as demonstrate their expertise with such species. Finally, investigators are not free to make a series of non-significant or significant changes to a protocol; a new protocol would have to be submitted in such a case.

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5. Justification of Numbers of Animals Required for Research

Animal Care and Use Committee of Wake Forest University

I. Sample Size:

There is no one method for sample size determinations or method for justifying the number of animals used in scientific research. The numbers of animals needed depend on the aims, designs, and measures used in the research. When an inferential statistic is used, the result of the power calculation used to determine the proposed number of animals should be provided. References to on-line guides to performing power calculations are found below. Listed below are some examples of sample size determinations or justifications for some hypothetical studies. These examples are not a template to be taken verbatim for actual research projects, but are provided as illustrations.

1. **Preliminary study - obtaining the variance required for power calculations:** This is a pilot study to develop new methods for measuring the abundance of a particular messenger RNA. There are no data to estimate the variability of the measurements by this new procedure. The mRNA levels will be measured in 12 animals in order to determine the variability in a population of animals. To allow for a potential 40% failure rate for technical reasons, a sample of 12-20 animals is required for this research.
2. **Hypothesis testing - comparison between 2 groups:** This research is designed to be able to compare catecholamine levels between rats 1 year of age and rats 5 years of age. Based on estimates of variability of catecholamine levels, a sample of 25 rats per group will be required to detect a 10% difference in catecholamine levels between the two groups, with 80% power at the 5% two-sided level of significance. In order to allow for a 20% inevaluability rate due to technical caveats, a total sample size of 62 rats is required for this research.
3. **Hypothesis testing - comparison to a known standard:** This research is designed to test if the prevalence of morbidity in immunocompromized rats is greater than 20%, the standard prevalence for normal rats reported by Smith and Jones, 1985. In order to be able to detect a true prevalence of 40% in the immunocompromized rats with 80% power at the 5% two-sided level of significance, a sample of 49 rats is required for this research.
4. **Estimating a proportion:** This study is designed to estimate the percentage of rats that will develop tumors after being injected with a tumor cell line. A sample of 45 rats is required to be able to estimate the percentage with 95% confidence intervals of $\pm 15\%$.
5. **Estimating a quantitative number:** This study is designed to determine the mean concentration of PDGF in nude mice. Based on a previous study by Smith and Jones, 1990, the estimated standard deviation of PDGF in normal rats is 10-micrograms/ml serum. A sample size of 18 is required to be able to measure a mean concentration of 175 micrograms per ml with a 95% confidence interval of ± 5 micrograms per ml (based on a confidence interval width of 1 standard deviation).
6. **Detecting prevalence:** This study is designed to detect the presence of the APP (Amyloid Protein Precursor) gene in the hippocampus of cynomolgous monkeys. A sample of 31 monkeys was chosen so that if the APP gene is not observed in the 31 monkeys, we will be 95% confident that the true prevalence of the APP gene is less than 10%.

Combination of 5 & 6: This research will initially study the presence of APP (Amyloid Protein Precursor) gene in a sample of 31 monkeys. An initial sample of 31 monkeys was chosen so that if APP gene is not observed in the 31 monkeys, we will be 95% confident that the true prevalence is less than 10%. If at least one monkey is observed to have the APP in the first 31 animals, an additional 14 animals will be used to be able to estimate the true prevalence rate with a 95% confidence interval of $\pm 15\%$.

7. **Descriptive anatomical studies:** Certain types of descriptive anatomical studies, including pathway tracing in the brain, immunocytochemical localization of various molecules, and *in vitro* autoradiographic receptor localization studies do not rely on quantification for the validation of useful data. In these cases, the number of animals required may be based on the experience of the investigator and similar types of studies published in the literature.
8. **Success of a desired goal:** Certain experiments require the use of animals in which the outcome is the success or failure of a desired goal. The generation of an antibody-producing cell line or the production of a transgenic animal by gene transfer are examples of such experimental objectives. Because the number of animals required is determined by the probability of success, the number of animals required can be difficult to estimate. Considerations based on the investigators experience or based on published accounts are required to provide estimates of the needed animals. These details should be provided in the justification. The Institutional Animal Care and Use Committee Guidebook from the Applied Research Ethics National Association/Office of Laboratory Animal Welfare (ARENA/OLAW) provides a good discussion on the complexities of creating transgenic lines.
9. **Breeding colonies:** The need to maintain a pool of genetically defined animals presents specific accounting problems. The minimal number of breeding pairs is determined by the experimental needs and by the breeding and rearing traits that may be associated with the particular genotype. Experimental groups should include breeding pairs and offspring, separated by genotype, if relevant. The animal numbers should include breeding pairs, projected litter sizes, and culled animals. Note that any animals that are culled before weaning are accounted for by a separate item in the protocol (Item #11C).

II. Harvesting of Animal Tissue

Some study designs require harvesting animal tissue that is subsequently used to perform experiments. Although the ACUC protocol form requires detailed description only of the methods relating to procedures performed on live animals, the number of animals required to harvest the tissue must be justified on the basis of the experiments that are performed on the harvested tissue. Sharing of other unused biological samples from these animals with other investigators is encouraged. Examples of animal number justification for studies involving cell culture of harvested tissue are found in the following.

Example 1. A biochemical study of a metabolic pathway in cultured hepatocytes is proposed. Cultured hepatocytes are prepared from the liver of a rat; the cultures are viable for one week. There are 10 groups of experiments that need to be performed on the cultured hepatocytes using various inhibitors of the pathway under study. Previous experiments determined a standard deviation of 19% in the activity of the metabolic pathway. The investigator needs to detect an effect of 25% on this pathway. Power calculation shows that 20 experiments per group (10 controls and 10 drug treatments per group) are required to detect this difference at the 5% level with a power of 80%. Thus, 200 successful experiments (10 groups x 20 experiments) are required for this study. Because experiments are sometimes unsuccessful due to technical difficulties, this number needs to

be increased to 220. At an assumed performance rate of 10 metabolic experiments per week, the experiments would be completed in 22 weeks and thus require 22 rats for the weekly preparation of cultured hepatocytes. To account for the occasional unsuccessful culture of hepatocytes (about 10%), 24 rats (22+2) are requested for the proposed study.

Example 2. The proposed study intends to examine growth rate and apoptosis in cultured myocytes. For the proposed experiments, a total of 10 timed, pregnant female rats are requested. Each female rat should deliver a litter of approximately 10 pups, based upon our prior experience using timed, pregnant female rats from Zivic-Miller. We will prepare both cultured myocytes and cardiac fibroblasts from the neonatal rat hearts, pooling the hearts from all of the pups in a single litter. Based upon previous studies in the literature (*full reference*), one 60 mm tissue culture dish or 1 cluster plate (either a 6, 12, or 24 well plate) of myocytes can be obtained per neonatal rat heart. Thus, from each litter of pups, 10 dishes of myocytes for individual experiments can be obtained.

In this pilot project, we request 2 litters of pups (2 adults and 20 pups) for initial studies, to learn the necessary techniques and obtain sufficient cells to characterize our preparations as to the percentage of myocytes per total number of cells (as an estimate of purity of the cultures). For the proposed experiments, a total of 8 litters of pups (8 adults and 80 pups) are requested. From the 10 available plates from each litter, 6 cluster plates will be used for growth assays (³H-thymidine incorporation, ³H-leucine incorporation, and cell number counts) and 4 plates will be used for the measurements of apoptosis (Annexin V and TUNEL assays).

Example 3. To characterize the response to 10 different angiotensin peptides, dose-response curves will be generated including a control and increasing doses of each peptide from 10⁻¹¹ to 10⁻⁴ M for a total of 9 different points, in duplicate, for each peptide (10 peptides x 9 concentrations x 2 duplicate samples or a total of 180 different determinations). To characterize the response to 5 different angiotensin peptide receptor antagonists with at least 5 of the 10 different peptides (assuming that 5 of the 10 proposed peptides cause an increase or a decrease in growth), experiments would include a submaximal concentration of the peptide, a 10-fold molar excess of the antagonist in the presence of the peptide, and a control with antagonist alone, in duplicate (3 experimental determinations x 5 angiotensin peptides x 5 peptide antagonists x 2 duplicate determinations or a total of 150 different determinations). For these studies we propose to use an n of 5 for each experimental determination. We have no prior experience with these exact types of studies in bone marrow cells making a power calculation difficult. However, based upon our previous experience measuring growth responses to angiotensin peptides in various types of cultured cells including vascular smooth muscle cells, astrocytes, and various cell lines, we anticipate that this will provide us with statistically significant results. Thus a total of 180 + 150 x 5 or 1650 individual determinations will be required to complete the proposed experiments.

From a single mouse, using both femurs, approximately 3 x 10⁷ total bone marrow cells can be isolated (based upon reported results in the literature). Based upon documentation from the manufacturer of the magnetic separation system that we propose to use, we anticipate a yield of approximately 1% Sca-1 cells from the total amount of bone marrow cells for a yield of 3 x 10⁵ Sca-1 cells. For each individual experiment, we anticipate that we will need 1.5 x 10⁴ Sca-1 cells per determination (per 1.2 ml of Methocult semisolid media selective for BFU-E growth (as indicated in the literature accompanying the Methocult growth conditions).

Thus, 20 determinations can be conducted with the bone marrow isolated from a single mouse. To provide sufficient cells for the proposed studies, we would need 82.5 mice. Based upon a potential cell contamination rate of 10% (during the isolation of bone marrow cells), we request a total of 90 mice for the proposed studies, over a two-year time period.

III. References

References (on-line references last verified May 22, 2004):

- <<http://www.graphpad.com/>>: This is the commercial site of the producers of GraphPad Prism, a self-contained statistical package for non-statisticians. They provide an excellent assortment of free on-line statistical calculators and have a lucid description of the principles of statistics that are especially relevant to biologists.
- <<http://davidmlane.com/hyperstat/power.html>>: A concise review of the basic principles of statistics, which includes a discussion of sample size calculations with links to sites where actual calculations can be performed and links to books and other resources.
- <<http://www.mc.vanderbilt.edu/prevmed/ps/>>: Access to a downloadable program by William D. Dupont and Walton D. Plummer, Jr. that runs only on MS Windows-based machines. The program can perform power and sample size calculations and permits the user to visualize consequences of changing the statistical requirements on the sample size.
- <<http://www.biomath.info>>: A simple site of the biomathematics division of the Department of Pediatrics at the College of Physicians & Surgeons at Columbia University. Provided by the authors of the article listed below.
- R. B. Dell, S. Holleran and R. Ramakrishnan. 2002. Sample size determination. *ILAR J.* **43**:203-212.

Entered January 5, 1995
Revised December 1998
Revised June 2002
Revised July 2002
Revised May 2004

6. Procedures for Handling Complaints Concerning Animal Care and Use

Animal Care and Use Committee of Wake Forest University

1. Receipt of Complaint by ACUC Chairman, ACUC member, or animal research concern line. A transcript will be made of complaint and filed in the Office of Research.

2. Determination of Seriousness of Complaint and Course of Action to be Taken
 - a. Executive decision as to disposition of complaint will be rendered by:
 1. ACUC Chairman and Vice-Chairman
 2. Attending Veterinarian
 3. Associate Dean for ResearchMeetings should be held with parties concerned to try to resolve the issues before a formal course of action is taken.

 - b. Courses of Action
 1. Minor concerns - handled by conferences with the Principal Investigator through ACUC
 2. Major concerns - initiation of a formal investigative process by ACUC

3. Initiation of a Formal Investigative Process
 - a. Appoint an ad hoc investigative committee from ACUC membership - ACUC Chairman

 - b. Establish a plan of action and timetable for completion of investigation - ACUC Chairman and Investigative Committee Chairman

 - c. Give official notification, course of action, timetable and list of charges to the Principal Investigator by all of the following methods:
 1. Memo - ACUC Chairman
 2. Personal communication in presence of Associate Dean for Research - ACUC Chairman
 3. Inform the Executive Dean, Principal Investigator's chairman and Associate Dean for Research by memo - ACUC Chairman

 - d. Inform legal counsel for the university - Office of Research

 - e. Inform person(s) issuing the complaint of the plan of action and timetable concerning the investigation. In case of minor complaints, also inform concerned party about course of action to be taken - ACUC Chairman

 - f. Contact Personnel Office to ensure no punitive actions are taken against person issuing complaint during the investigative process - Office of Research

- g. Inform Public Relations and Marketing about impending investigation to prepare response should investigation generate questions from the media. Advise all involved to refer media questions to them - Office of Research
 - h. The ACUC has the responsibility and authority, depending on nature of charges, to require that all new research related to the complaint be halted by the Principal Investigator until formal investigation is complete. All ongoing research should be closely monitored.
4. Investigative Process - Ad Hoc Committee Action
- a. Determine the validity of the charges by interviewing:
 - 1. Person(s) making the charges
 - 2. The Principal Investigator charged
 - 3. Expert witnesses, technicians, students, ARP personnel, etc.
 - b. Determine if any violations of regulations or breach of ethical standards have been committed. If so, formally list them. Was complaint against procedures approved by ACUC?
 - c. Recommend an appropriate course of action to be taken with respect to the Principal Investigator and the person making the complaint.
 - d. Submit a formal written report to the ACUC Chairman in a timely manner (usually no more than one month) to include:
 - 1. Validity of the charges
 - 2. Violation of specific regulations and/or breach of ethics
 - 3. Recommended courses of action, which may include:
 - a. Discontinuation of research
 - b. Requiring Principal Investigator to adopt new rules, regulations and/or guidelines and suggestions, mechanisms for enforcement
 - c. No further action required, e.g., charges unsubstantiated, etc. Disciplinary action may be taken if charges are found to be malicious or intentionally dishonest.
5. Course of Action - ACUC Action
- a. Receipt and distribution of Ad Hoc Committee report - ACUC Chairman
 - b. Report to full Committee - Ad Hoc Committee Chairman
 - c. Recommendation(s) presented - Ad Hoc Committee Chairman
 - d. Committee discussion and vote - ACUC Chairman
 - e. ACUC Chairman communicates findings to Institutional Official
6. The Institutional Official will review the findings of the Ad Hoc Committee and take whatever appropriate action required, e.g., notification of the sponsor of the research, Principal Investigator, complainant, department Chairman and ARP Director.

2-15-95; re-issued 12/98; revised 5/18/04

7. Occupational Health Program For Personnel

Animal Care and Use Committee of Wake Forest University

The Occupational Health Program for Personnel Caring For or Using Laboratory Animals (OHPLA), administered by the Medical Center Employee Health Services (MCEHS), consists of education including information on zoonotic diseases, baseline and periodic medical evaluations, and provisions for treating illness or injury. It is designed to protect both personnel and laboratory animals. The principal concern is to protect personnel from and monitor exposure to hazards emanating from animals themselves (i.e. infectious agents, bites and allergies) as well as exposure to secondary hazards such as radiation and toxic chemicals. The specific elements of the program vary depending on the nature and extent of an employee's exposure to animals.

Eligibility

Personnel of Wake Forest University Baptist Medical Center (WFU, School of Medicine, and North Carolina Baptist Hospitals, Inc.) are eligible for inclusion in this program if they have:

- Any direct contact (touch) with vertebrate animals and/or their tissues or excrement
- Any potential aerosolized exposure (in same room/area) to the following diseases:
 - 1) Tuberculosis through exposure to nonhuman primates, or
 - 2) Q-Fever through exposure to sheep
 - 3) Rabies through exposure to bats

Mechanism For Enrollment

All eligible paid and unpaid, temporary, part-time, and full-time employees of Wake Forest University are enrolled in the Occupational Health Program at the time of employment or position transfer. Employees and their supervisors are required to designate whether their duties make them eligible for enrollment in the Occupational Health Program. Those eligible are required to report to the MCEHS as a part of the pre-placement orientation. In addition, all personnel listed on an Animal Care and Use Committee protocol are required to enroll before the protocol will be approved.

Program Components

The Occupational Health Program consists of education, a pre-placement medical questionnaire and health evaluation, pre-exposure immunizations, periodic health evaluations during the period of exposure, and evaluation and treatment of injuries and illnesses related to laboratory animal exposure. The components of the health evaluation are based on the functional requirements of the position, the type of animal(s) contacted, and the individual's prior medical history. Specific elements of the program, including specific tests depend on the species of animals as well as the nature of exposure and the procedures used. The table in Appendix IX outlines the elements of the program by species exposure.

Principal investigators, course masters, or laboratory/unit directors are responsible for assessing the specific risks, establishing procedures to minimize these risks, and assuring the safe conduct of their experiments employing experimental animals. The responsible faculty member must be aware of the types of health risks and appropriate control measures associated with the specific research procedures and species used. When required, consultation on these matters is sought from MCEHS and/or other professionals within or outside the University.

See Appendix IX for requirements by species

Reviewed 5/18/04

8. Photography/Videotaping Within Animal Facilities, Research and Teaching Laboratories

Animal Care and Use Committee of Wake Forest University

Wake Forest University maintains an open policy regarding reasonable requests to visit its animal facilities, research and teaching laboratories. However, in order to protect the confidentiality of faculty research, to provide a minimally disruptive atmosphere for the residing animals and to guard against the misinterpretation of appropriate and humane policies and procedures, photography is not allowed except for official purposes that are approved as such by the Chair, Animal Care and Use Committee, ARP Director, Public Relations Representatives, and other officials, depending upon circumstances.

I. ACUC Guidelines For Administering The WFU Policy

A. Government or private Inspector

An inspector conducting a site inspection who desires to make photographs must agree to the following conditions:

1. Photographs may be taken only as an official part of the inspection and used only for documentation of items that cannot be documented in writing.
2. Prints of photographs used in a report must be provided to WFU for review and approval, as are all other components of site inspection reports.
3. All negatives and prints of photographs not used in an official report will be destroyed.
4. Photographs will not be distributed or used in any way other than as supporting evidence for an official site inspection report.
5. When photographs are used to document deficiencies, equivalent photographs taken by WFU also be used to document corrections and both will be used together in any report of ARP compliance with the Animal Welfare Act (AWA) provision.
6. Failure to comply with the above provisions will be considered by WFU as a breach of due process in USDA enforcement of the AWA and subject to legal redress.

The inspector must sign a copy of a document that includes the above 6 points (see appendix) as evidence that the policy was clear prior to use of photographs.

B. General Public

The Animal Resources Program Director or his designee must accompany members of the general public when a tour of the animal facilities is conducted. The respective department chairman, principal investigator or course/master must supervise visits to research/teaching laboratories where animals are involved. Photographs may not be taken within the animal facilities, research or teaching laboratories.

C. Professional Visitors/WFU Faculty

Those scientists/officials visiting the institution for professional reasons involving collaboration or information exchange may be allowed to take photographs that relate to the nature of their visit. The approval for such photography will rest with the professional judgement of the WFU faculty

member who serves as host. WFU faculty may take pictures relating to the documentation for their own research/teaching programs.

D. News Media

Requests from the news media to photograph animal facilities, research or teaching laboratories will be handled by the Information and Publications Office through the ACUC and ARP as they arise.

E. Other Exceptions

Requests for exceptions to this policy must be approved by the ACUC and authorized by the Dean.

II. Videotapes of Animal Research

In 1987, an incident occurred at the East Carolina School of Medicine in which a videotape used to teach first year medical students animal surgery techniques was copied and acquired by an animal rights group for display at a news conference.

The tape was edited and displayed out of context and presented a distorted picture of the institution's use of animals in teaching and research. To avoid such a destructive incident at our institution, the ACUC asks that all faculty who have or will have made videotapes of their research animals take the following precautions:

1. Store tapes in a secure, locked area with very limited access.
2. Do not authorize copies of tapes unless supervised by you personally.
3. Erase immediately all tapes no longer in use or of value to your research.
4. Keep a checkout list including dates and circumstances of those persons borrowing your tapes. Even under these circumstances access should be very limited and closely supervised.

F. Permission Form for photography/video is in Appendix I

1-5-95

re-issued 12/98
reviewed 5/18/04

9. Research using Animal Materials Obtained from Other Institutions

Animal Care and Use Committee of Wake Forest University

Collaborative research with investigators from other institutions occurs with increasing frequency. When the collaboration involves the receipt of and experimentation on live animals, the current guidelines require an approved ACUC protocol at Wake Forest University. However, situations in which animal **tissues or body fluids** may be sent to investigators at WFU for experimentation or analysis are less clearly covered.

General guidelines

1. If animal **tissues or body fluids** are sent to WFU investigators only for analysis or *in vitro* experimentation, then a WFU ACUC protocol for these experiments may not be required. However, there are several caveats. If you request extra treatment of the animals before obtaining samples, such as surgical or pharmacological intervention that was not part of the original protocol at the supplying institution, then these animals should be included in an ACUC protocol here. On the other hand, if you collaborate on measurements using animal **tissues or body fluids** from experiments that were designed and conducted by outside investigators, then a WFU protocol is not required. Even in this case, the ACUC recommends that investigators assure themselves of proper animal welfare and comply with guidelines for publication by requesting from the collaborating investigator(s) the Animal Welfare Assurance number of the other institution and the title and IACUC approval date of the project.
2. If you contract with another institution or company to raise animals for research or to supply animal **tissues or body fluids** to you for a fee, then the outside institution becomes an extension of your research and must be described and approved in an ACUC protocol. This does not apply in the event that tissues or body fluids are obtained or purchased from commercial sources that provide tissues on a routine basis.
3. If animal materials are sent to WFU from another institution for introduction into live animals here, such as a tumor cell line, then an ACUC protocol must be submitted for these experiments.
4. Other examples of situations in which a WFU protocol is *not* required include tissue obtained from slaughterhouses, chick embryos before hatching, excess biopsy samples from live animals when the samples would otherwise have been discarded, and post mortem tissues unless you required ante mortem modification of procedures.

Revised 06/07/04

10. Multiple Survival Surgeries On A Single Animal

Animal Care and Use Committee of Wake Forest University

In deciding whether to permit multiple survival surgeries, the ACUC must weigh two ethical imperatives that in practice often conflict with each other. The first is that painless (or at least less painful) deaths are ethically superior to painful deaths. The second imperative is to kill as few animals as possible (that is, reduction). In some cases, reducing the number of animals used can mean increasing the pain caused to an individual animal. Conversely, reducing the pain to individuals can mean killing more animals.

Decisions about multiple survival surgeries are made even more complex by an array of other issues, including:

- a) the nature of the proposed surgeries and their potential to cause pain or disability,
- b) uniqueness of the model,
- c) the scientific value of the model,
- d) the difficulty in producing the model,
- e) the availability of the model,
- f) the availability of the particular species under study.
- g) not all meritorious scientific justifications are, by themselves, compelling,

It is the responsibility of the ACUC to decide whether or not proposals for multiple survival surgeries are appropriate and to describe in writing how the decision was made. Taking the points noted above into consideration, the guidelines outlined below comply with the Animal Welfare Act and USDA Animal Care Policy #14; <http://www.aphis.usda.gov/ac/policy/policy14.pdf>.

1. Protocol requesting multiple survival surgery that may combine one or more minor survival surgery and one or less (lifetime) major survival surgery

- A. Whenever possible, all operative procedures should be done at one time to minimize post-operative discomfort and distress to the animal.
- B. In experiments requiring multiple survival surgeries, the justification for the additional surgeries should be related to the original procedures. That is, if an original set of procedures creates a unique model or scientific opportunity, it may be justifiable to perform additional procedures on the animal to take scientific advantage of those kinds of opportunities. Additional surgeries would be difficult to justify, however, if they were not scientifically related to the original procedures.
- C. In some cases, it might not be perfectly clear whether or not the scientific rationale is sufficient to justify additional survival procedures. Other factors that might be considered include the nature, severity, and difficulty of recovery from the proposed surgeries, the extent of disability caused by the surgery, the uniqueness of the model, the difficulty in producing the model, the availability of the model, and the availability of the particular species on which the model is based. Thus, given equally strong scientific rationales, it

might be considered more reasonable to perform three or four scientifically related survival surgeries on a set of monkeys that has received an ovariectomy, several years of high fat diets, and drug therapy than on a set of rats that has had a similar history in relation to its life span. The ability to replace the rats to perform additional experiments far exceeds the ability to replace the monkeys. The monkeys are more difficult and expensive to obtain, and several years of effort would be required to create the appropriate model. Even then, it is often more powerful to correlate data sets obtained from individual subjects than to compare data across subjects. In addition, the ethical cost of killing additional monkeys might be considered too extreme in relation to the cost of killing an equal or greater number of rats.

D. Specific guidance to investigators using colony animals:

Because we deal with monkeys that might be returned to a colony after a set of survival surgeries, it is imperative that the committee be apprised of the lifetime research history of any animals slated to enter a new study after they have been involved in previous studies (including research performed at other institutions). It is the responsibility of the principal investigator to consult with the attending veterinarian to identify such animals and notify the ACUC of their history so that this factor may be taken into account in evaluating the protocol for the proposed study.

2. Original protocol requesting multiple major survival surgery

The Animal Welfare Act considers multiple major survival surgeries acceptable only if they can be justified scientifically. Cost and convenience, by themselves, cannot be considered as sufficient justifications.

USDA/Animal Care – Policy #14

“No animal assigned to a proposal is to be used in more than one major survival operative procedure unless the multiple procedures are included within one proposal, justified for scientific reasons by the Principal Investigator, and preapproved by the Institutional Animal Care and Use Committee (IACUC). However, an animal that has an emergency major operative procedure as part of proper veterinary care may still be used in a proposal that requires a major survival operative procedure.”

3. Future protocol requesting additional major survival surgery

The Animal Welfare Act prohibits additional major survival surgery on an animal in future protocols, unless the investigator is granted an exemption through the USDA.

USDA/Animal Care – Policy #14 (continued)

A major survival operative procedure must not be performed a second time on an animal in a separate proposal. In order to comply with the intent of the Animal Welfare Act (AWA), animals surviving a major operative procedure must be identified (written documentation) to prevent their use in a second major survival operative procedure.

The AWA and its regulations allow an exemption to limiting animals from being used in only one proposal with a major survival operative procedure. The Institutional Official

of the research facility should make the exemption request to the appropriate Animal Care Regional Director, who forwards it to the Animal Care Assistant Deputy Administrator for review and recommendation to the Deputy Administrator. The request for exemption should include the following information:

- a. An outline of the research proposals for which the procedure is requested*
- b. The species and the approximate number of animals involved in the exemption request*
- c. The time frame for the proposed exempt procedure*
- d. The number of major operative procedures to be performed on a given animal, the frequency of such procedures, and the period of time between each major operative procedure*
- e. Measures to be taken to ensure that pain/distress are minimized*
- f. A complete justification for the exemption in which cost is not normally a major criterion*
- g. An assurance that all other stipulated requirements of the AWA and regulations will be met in consideration of this exemption*
- h. An assurance that the facility's IACUC has approved the exemption.*

The Animal & Plant Health Inspection Service (APHIS) may respond to the formal request by approving the request as written, granting a portion of the request, imposing additional limitations, or denying the request. An annual IACUC evaluation of the exemption is required, which consists of an IACUC assessment of the animals and the effectiveness and soundness of the methods and procedures used. This information is to be included in the report of the IACUC functions. Considerations for the renewal or continuation of the exemption will be based on the IACUC's recommendations following their review. The exemption must be included in the Annual Report (APHIS Form 7023).

Revised 07-21-04

11. Use of Inhalation Anesthetics in an Open Chamber

Animal Care and Use Committee of Wake Forest University

The open-drop (anesthetic chamber) method for inducing anesthesia in laboratory rodents has been approved by the institutional Animal Care and Use Committee under the following conditions:

1. The closed anesthetic chamber should contain a raised floor to protect the animal from direct contact with the anesthetic agent, which is usually soaked in gauze. Alternatively, the soaked gauze may be placed in a separate container that allows the gas to permeate but prevents any direct contact of the material with the animal.
2. The chamber must be used in a chemical fume hood to exhaust the anesthetic vapors.
3. Isoflurane dispensers fed to an anesthetic chamber are acceptable provided that the chamber is placed in a chemical hood or vented through the vacuum system.

The best anesthetic for this system is either halothane or isoflurane. Halothane is the most effective inhalant anesthetic for euthanasia.

Use of Ether as an Anesthetic or Euthanizing Agent

The use of ether as an anesthetic or euthanizing agent is no longer an approved agent under any circumstances.

12/98

revised 07/2004

12. Decapitation and Cervical Dislocation as a Form of Euthanasia in an Unanesthetized Animal

Animal Care and Use Committee of Wake Forest University

Appropriate methods of euthanasia are an important component of an effective Animal Care and Use Committee program. In most cases we defer to the policies described in the 1993 report of the AVMA panel on euthanasia. However, we believe that this report is not complete in dealing with the use of decapitation as a method of euthanasia. Decapitation without anesthesia shall be an appropriate form of euthanasia for small mammals, including mice, rats, guinea pigs, and small rabbits in those situations in which the use of anesthetics would obscure the dependent measures of interest. Cervical dislocation is appropriate for euthanasia of avian species, mice, and immature rats (<200 g) and rabbits (<1 kg).

Decapitation and cervical dislocation should be performed after the animal has been lightly anesthetized unless the anesthetic would obscure the dependent measures of interest. To conform with our policy, decapitation and cervical dislocation without prior anesthesia must be scientifically justified based on published data and carried out by trained personnel, and must be part of an approved protocol. Some manipulative skills and experience are required for decapitation to be carried out in a humane manner; therefore, the ACUC should be provided with assurance that the investigators are trained to use this procedure.

Procedures should be executed as rapidly and painlessly as possible. Every attempt should be made to perform either of these methods of euthanasia on animals such that it minimizes reactions among other living animals (i.e., performed in a room separate from the housing area). These procedures should also be performed out of the presence of individuals who are not directly familiar with the procedure, except in the context of training. In the training of new personnel, where possible these procedures should be practiced on carcasses or on anesthetized animals until proficiency in the technique is established.

A guillotine is recommended for decapitation of avian species, rodents, and small rabbits. A sharp scissor of sufficient blade length to completely transect the neck is permissible to use for decapitation of small animals such as neonatal rodents and small mice. The instrument used in a decapitation procedure must be properly designed to insure decapitation in a uniformly instantaneous manner. It should be cleaned of blood and tissue between each successive use.

Appropriate members of the Animal Care and Use Committee of Wake Forest University should be contacted prior to protocol submission to discuss specific experimental requirements that do not strictly conform to these recommendations.

Procedure for cervical dislocation:

1. Lightly sedate or anesthetize the animal according to the protocol;
2. Restrain the rodent with ventral body surface against a firm, flat surface and grasp the tail with one hand;

3. Place a stick-type pen, a rod-shaped piece of 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;

4. To produce the dislocation, quickly push forward and down with the hand or object restraining the head while pulling backward and up 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. Confirm respiratory arrest, and verify by palpation that there is no heart beat.

Procedure for decapitation:

1. Lightly sedate or anesthetize the animal according to the protocol;

2. If using a guillotine, position the animal in the guillotine so that the cutting blades are below the occipital condyles, and will contact the region of the cervical vertebrae. Perform the procedure using a firm, instantaneous motion. If using scissors, ensure that the scissor blades are long enough to transect the neck in one movement.

Revised 7/20/04

13. Post-surgical Medical Care Procedures

Animal Care and Use Committee of Wake Forest University

The Animal Care & Use Committee (ACUC) and the Animal Resources Program (ARP) have the responsibility to ensure that post-surgical care for research animals is administered in accordance with current professional and regulatory guidelines; however, the ultimate responsibility for the provision of post-surgical care lies with the principal investigator. The veterinary faculty of the ARP, veterinary residents, veterinary postdoctoral trainees and animal health technicians also play an active role in postoperative care. A veterinarian and animal health technician make daily observations of all non-rodent animals including postoperative animals and work closely with the principal investigator to ensure appropriate veterinary care is administered. The principal investigator, the principal investigator's technician, an ARP veterinarian, or an ARP animal health technician administers specific treatment for observed conditions. The WFU Attending Veterinarian has the final authority with regard to the treatment or euthanasia of experimental animals. Following daily observations, the ARP veterinarians and staff are available for consultation or assistance.

For any survival procedure on non-rodent species, the ARP form titled "Surgical Chart" (Appendix II) must be completed in its entirety. This information provides useful and necessary data to assist the ARP clinical staff and is required for regulatory inspections.

The surgical chart requests (provides the option for) the use of preemptive analgesia in addition to post-operative analgesia. Preemptive analgesia refers to the administration of a drug with analgesic activity *before* a surgical procedure. The use of analgesics in this manner is encouraged as it has been shown to be an effective way to reduce post-operative pain following many types of surgical procedures. Drugs that provide preemptive analgesia include the opioids (agonists, agonists-antagonists), alpha-2 agonists, non-steroidal anti-inflammatory drugs, and local anesthetics. Anesthetic drugs that induce and maintain general anesthesia do not induce preemptive analgesia.

In addition, a post-surgical record must be kept in proximity to the animal following recovery. Post-operative observations must be recorded on the ARP form titled "Post Surgical Medical Chart" (Appendix III). This form must be completed in its entirety and continued until the animal reaches stage 0. It should also include the findings of each physical examination during the recovery period. The post-surgical medical record will be retained as a part of the animal's permanent medical record. It is recommended that experimental notations be kept in a separate location.

The ANIMAL WELFARE ACT as amended (7 U.S.C. 2131 ET. Seq.) states in section 13 (3)(A) that the appropriate use of anesthetic, analgesic or tranquilizing drugs are to be administered to animals in experimental procedures that cause pain or distress. Also, the *Guide for the Care and Use of Laboratory Animals (NRC 1992)* says that the proper use of anesthetics and analgesics in research is ethically and scientifically imperative. To ensure compliance with these guidelines, the forms in appendix II and III request the attending personnel to assign a pain score pre-operatively and post-operatively. The pre-operative score will determine a baseline for the animal's behavior and the post-operative score will assist the attending personnel in evaluating whether or not the animal is in a painful state and to what degree. To assign a pain score, refer to the ARP form titled "Pain Score Evaluation Form" (Appendix III).

For purposes of medical monitoring, it is helpful to describe stages of recovery from surgery and anesthesia. Animals should be examined and the findings recorded according to the staging system outlined below. This staging system is designed for investigators utilizing rabbits, carnivores, primates, and hoof stock. Whereas rodents are exempt from this organized scheme, it is imperative that these species also receive adequate post-surgical care.

A. Stage 4 – Animal unconscious or semi-conscious

(Indicators of an animal in Stage 4 of anesthetic recovery: The animal lacks a reasonable response to exogenous stimuli, cannot lift and maintain an elevated head position, and is at a level of anesthesia that would not allow a normal, healthy animal to maintain sternal recumbency.)

1. Examine and record findings no less frequently than every 30 minutes. The animal should be given continuous attention throughout stage 4. Examples of notations include:
 - a. Body temperature
 - b. Heart rate or pulse
 - c. Respiratory rate
 - d. Time of extubation
 - e. Comments of general condition
 - f. (Dogs and Cats) Capillary refill time (record in seconds)
2. Unless the type of surgery conducted (i.e. thoracotomy) precludes it, rotation from one lateral recumbency to the other should occur every 30 minutes to prevent dependent pulmonary congestion and edema.
3. Ambient temperature should be adjusted (heat lamp or warming board) to bring body temperature to normal. Care must be taken to not burn or overheat animals at this stage of recovery. Animals should be kept dry.
4. The state of hydration should be assessed and fluids should be provided, as necessary.
5. Give analgesic medication as specified in the ACUC protocol.

B. Stage 3 – Animal is conscious

(Indicators of an animal in Stage 3 of anesthetic recovery: The animal responds to exogenous stimuli, can lift and briefly maintain an elevated head position, and is at a level of anesthesia that generally would allow a normal, healthy animal to maintain sternal recumbency but not stand.)

1. Examine and record findings no less frequently than every 6 to 10 hours depending on the nature of the surgery and the status of the animal. More frequent examinations may be necessary if the status of recovery requires it.

Examples of notations include:

- a. Comments of general condition
- b. (Dogs and Cats) Body temperature until the animal can maintain normal body temperature, respiration rate, heart rate, estimated state of hydration, etc.

c. (Dogs and Cats) Capillary refill time

2. Examine closely for other abnormalities.

3. Keep the animal dry and adjust the ambient temperature to bring the body temperature to normal.

4. Give analgesic medication as specified in the ACUC protocol.

C. Stage 2 – Animal can stand, maintain balance and move about.

1. Examine daily or more frequently if appropriate and record findings. Examples of notations include:

a. Body temperature

b. Hydration

c. Attitude (alert or depressed)

d. Activity (active or inactive)

e. Food consumption

f. Water consumption

g. Condition of operative site

2. Examine closely for other abnormalities.

3. Give analgesic medication as described in the ACUC protocol.

D. Stage 1 – Animal is active, alert, eating and drinking normally; skin sutures are in place.

1. Examine daily and keep a post-surgical record of surgical site care until the sutures are removed.

2. Sutures must be removed within 10 to 14 days of surgery unless scientifically or clinically justified and approved by the ACUC. In the case of non-human primates housed in group pens, absorbable sutures can be used to close skin wounds. If absorbable sutures are used, records must be continued for 14 days post-operatively. After 14 days, the records maybe closed if there are no complications; however, any external suture-remnants must be removed the next time the animal is sedated.

E Stage 0 – Animal is normal and skin sutures removed.

1. Specific post-surgical care and record are no longer required.

If progress from Stage 4 to Stage 0 is not as expected or if there are medical complications, the ARP veterinary staff must be contacted for assistance. Emergency phone numbers are located by the phones in animal facilities.

4/18/02

14. Use of Hypothermia for Anesthesia in Mammals

Animal Care and Use Committee of Wake Forest University

Hypothermia is an acceptable form of anesthesia for the preweanling animals (<2 weeks of age) listed below:

Rodents
Rabbits
Marsupials (including Opossum)

Adult animals, juvenile animals that have been weaned and precocious newborn rodents (e.g. guinea pigs) are specifically excluded.

Three alternative procedures are acceptable for inducing anesthesia.

a) The preweanling animal is placed directly on a gauze covered bed of crushed ice. This method may be complicated by skin freezing at the point of contact with ice that is below 0°C. A gauze cover over the ice will help to minimize skin freezing.

b) The animal is placed inside a glass test tube which is immersed in ice. The time of onset of lethargy may be considerably slower than in the other methods.

c) The animal is immersed in iced water, with care being taken to avoid water aspiration and drowning.

In all 3 methods the end point indicating the readiness of the animal for a procedure is lethargy and immobility. The animal can be expected to remain immobile for up to 10 minutes. If additional time is needed for the procedure, immobility may be maintained if the animal's temperature is kept low using a cold pack. No additional anesthesia or analgesia is required.

Postoperatively the animals are warmed to normothermia by placing them in an incubator at 33°C. Placing the animal on a heating pad or under a heat lamp is strongly discouraged as it may lead to tissue damage. The animal should be mobile and able to return to its cage in 20-30 minutes.

Reference: Phifer, C.B. and Terry, L.M. Use of hypothermia for general anesthesia in preweanling rodents. *PHYSIOL BEHAV* 38 887-890, 1986.

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15. Use Of Freund's Adjuvant in Laboratory Animals

Animal Care and Use Committee of Wake Forest University

The Animal Care and Use Committee (ACUC) and the Animal Resources Program (ARP) have developed the following guidelines for the use of Freund's adjuvant in an attempt to eliminate, or reduce to a minimum, animal discomfort associated with the use of this agent. **Consideration for departure from these guidelines requires written scientific justification to the ACUC.**

Freund's Complete and Incomplete Adjuvants cause local inflammation at the site of injection, often resulting in painful lesions. Before using these or any other inflammatory adjuvant, consideration must be given to the use of non-inflammatory alternatives such as ethylene-vinyl acetate copolymer, ribi adjuvant system, muramyl dipeptide and liposomes. In many cases, non-inflammatory Adjuvant have been shown to produce antibody titers comparable to those induced by inflammatory adjuvant (1,2,3).

General Guidelines

1. Freund's Complete Adjuvant (FCA) should only be used for the first (priming) dose. Subsequent doses of antigen-adjuvant complex should be made using Freund's Incomplete Adjuvant (FIA).
2. The ratio of antigen to adjuvant should be 1:1. If FCA is used, the concentration of mycobacteria should not be more than 0.1 mg dry bacterial mass per ml. The use of formulations containing higher concentrations of mycobacterium has been associated with proportionally increased amounts of inflammation and necrosis (4). Commercial preparations containing more than the acceptable number of mycobacteria per ml can be diluted to the approved concentration with FIA.
3. Acceptable routes of administration include subcutaneous and intramuscular injection in all species and intraperitoneal administration in mice. Any other route of administration requires written scientific justification to the ACUC based on published data.

In all species, intradermal injection of FCA or FIA has been associated with a high incidence of necrotizing dermatitis and tissue sloughing (4,5). Intradermal injection of Freund's adjuvant must be justified to the ACUC.

The use of FCA or FIA in the footpad or toepad of rabbits and rodents has been associated with a high degree of pain and distress (4,5). Footpad or toepad injection of Freund's adjuvant must be justified to the ACUC.

FCA should never be given intravenously. Acceptable routes of administration are listed below for each species in Species Specific Guidelines.

4. The volume of antigen-adjuvant complex injected has been shown to be a major determinant of the degree of inflammation and pain that results when FCA and FIA are used in animals. The appropriate volume varies with the species used and the route of administration. Acceptable volumes are provided in Species Specific Guidelines (see below).

5. Whenever possible, the sites of injection should be widely separated to prevent individual lesions from coalescing which often results in disruption of local blood supply and contributes to cell death and tissue sloughing.

6. The interval between first, second and subsequent doses of antigen-adjuvant complex should be determined by the antibody response of the animal but should be maximized whenever possible. If pain and discomfort follow injections, the interval between subsequent injections should be of sufficient length to allow a recovery period.

7. The injection site must be observed by the investigator or his/her designate, a minimum of three times/week, for four weeks after each injection. If a lesion develops at the injection site, it must be reported through established channels, e.g. ARP personnel, and receive appropriate veterinary care. Such lesions must be inspected at least three times per week by the investigator or his/her designate, until all lesions are healed.

Species Specific Guidelines

Provided below are guidelines for the use of FCA or FIA in each animal species listed (1-7). Deviation from these approved guidelines requires written scientific justification to the ACUC.

1. Mice and other small rodents:

Approved Routes and Volumes (maximum volume/site):

- Subcutaneous, usually in neck or back region, 0.10 ml/site,
- Intramuscular, in biceps femoris muscle, 0.05 ml/site,
- Intraperitoneal (7), 0.10 ml/injection.

2. Rats:

Approved Routes and Volumes (maximum volume/site):

- Subcutaneous, usually in neck or back region, 0.10 ml/site,
- Intramuscular, in biceps femoris muscle, 0.05 ml/site,

3. Guinea pigs:

Approved Routes and Volumes (maximum volume/site):

- Subcutaneous, usually in dorsal neck region, 0.40 ml/site,
- Intramuscular, in biceps femoris muscle, 0.10 ml/site,

4. Rabbits:

Approved Routes and Volumes (maximum volume/site):

- Subcutaneous, usually in interscapular or back region, 0.25 ml/site,
- Intramuscular, biceps femoris muscle, 0.50 ml/site,

5. Goats and other large animal species:

Approved Routes and Volumes (maximum volume/site):

- Subcutaneous, usually in neck and leg regions, 0.1 ml/site,
- Intramuscular, biceps femoris muscle, 1.0 ml/site, no more than two sites per muscle.

Notes: FCA should not be used in horses.

6. Nonhuman primates:

Notes: FCA should not be used in nonhuman primates.

REFERENCES:

1. Niemi SM, Fox JG, Brown LR, et al. Evaluation of ethylene-vinyl acetate copolymer as a non-inflammatory alternative to Freund's adjuvant in rabbits. Lab Anim Sci 1985;35:609-612.
2. Bomford R. The comparative selectivity of Adjuvant for humoral and cell-mediated immunity. I. Effect on the antibody response to bovine serum albumin and sheep red blood cells of Freund's incomplete and complete Adjuvant, alhydrogel, Corynebacterium parvum, Bordetella pertussis, muramyl dipeptide and saponin. Clin Exp Immunol 1980;39:426-434.
3. Woodard LF. Adjuvant activity of water-insoluble surfactants. Lab Anim Sci 1989;39:222-225.
4. Broderson JR. A retrospective review of lesions associated with the use of Freund's adjuvant. Lab Anim Sci 1989;39:400-405.
5. Amyx HL. Control of animal pain and distress in antibody production and infectious disease studies. J Am Vet Med Assoc 1987;191:1287-1289.
6. Canadian Council on Animal Care. CCAC Guidelines on Acceptable Immunologic Procedures. 1989.
7. Toth LA, Dunlap AW, Olson GA, et al. An evaluation of distress following intraperitoneal immunization with Freund's adjuvant in mice. Lab Anim Sci 1989;39:122-126.

1-5-95

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16. Use of Animal Death as an Endpoint in a Study

Animal Care and Use Committee of Wake Forest University

Some studies are designed such that death of an animal determines the endpoint of data collection. An example of such an experimental design is the LD50 test, in which the dose of an agent is determined to cause mortality in 50% of a cohort of animals. LD50 studies are usually unacceptable and rarely are required to complete an experimental design. Animal death as an endpoint to a study has the potential to cause substantial pain or distress. Therefore, considerable effort must be made to employ an endpoint other than death.

If an investigator believes there are no alternatives to death as the endpoint, the study design will be approved only if scientifically justified.

Animals used in approved studies involving death as an endpoint must be monitored as described in the document A Policy on Minimizing Pain/Distress in Rodents.

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reviewed 9/04

17. Minimizing Pain/Distress in Rodents

Animal Care and Use Committee of Wake Forest University

The Wake Forest University ACUC recognizes that there may be a legitimate need to conduct some experiments which have the potential to cause significant pain and/or distress for rodents. Examples include tumor biology studies, cancer studies where different treatment modalities are being evaluated, 50 assays, infectious disease studies and experiments involving the production of significant fluid. Approval is limited to those protocols in which scientific justification is provided and criteria are met for monitoring rodents at risk of experiencing pain and/or distress.

In general, the ACUC requires specific intervention (i.e. administration of analgesics/tranquilizers, euthanasia) at a point significantly before pain/distress is experienced or death occurs. Studies in which there is potential for a rodent to experience significant pain/distress prior to death must be scientifically justified. Investigators must explain why animals cannot be euthanized prior to the development of debilitation and/or why analgesics/tranquilizers cannot be administered to alleviate pain/distress. The ACUC may request additional information or seek outside consultation when evaluating the justification. If adequate justification is not provided, the investigator will be required to identify and utilize earlier endpoints for euthanasia, or to administer analgesics to alleviate pain associated with the experimental manipulation.

Guidelines

The following guidelines must be used in experiments to avoid rodents experiencing significant pain and/or distress prior to death.

1. Frequency of Monitoring

Rodents should be monitored at least once daily, including weekends and holidays, for signs of deterioration. Once signs of deterioration are observed, rodents should be monitored at least twice daily until they are euthanized. Animals showing signs of end-stage illness should be euthanized immediately. Signs of deterioration and end-stage illness in rodents are provided in 3 below.

2. Personnel Training

The principal investigator is responsible for assuring that personnel are trained to detect signs of deterioration and end-stage illness. Upon request, training is available from the Animal Resources Program (ARP) veterinary staff. An ARP veterinarian must be consulted if there are questions or concerns about the health status of a specific animal. The WFU Attending Veterinarian has final authority in situations where differences of opinion exist regarding an animal's health status.

3. Criteria for Monitoring Rodents

a. Signs of Deterioration

(Rodents exhibiting the following signs must be monitored at least twice daily, including weekends and holidays.)

Mice

Posture: Hunched, sleeping posture

Appearance: Piloerection, weight loss, dehydration, sunken abdomen

Mice (cont.)

Behavior: Initially timid and apprehensive, becoming aggressive; later becoming quiet, unresponsive and non-social

Activity: Decreased food and water intake, reflex withdrawal, biting at source of pain or affected area, increased activity, self-mutilation

Locomotion: Unsteady gait, lameness

Eyes: Sunken, with or without discharge

Respiration: Increased respiratory rate, labored breathing, chattering, nasal discharge

Rats

Posture: Recumbent, hunched with head tucked into abdomen

Appearance: Piloerection, weight loss, dehydration

Behavior: Initially aggressive progressing to depression and unresponsiveness

Activity: Decreased food and water intake, reduced exploratory behavior, aversive or aggressive behavior towards cagemates, self-mutilation

Locomotion: Stilted gait, lameness

Eyes: Almost closed, sunken, red-stained discharge

Respiration: Increased respiratory rate, sneezing and red-stained nasal discharge

b. Signs of End-Stage Illness in Rodents

(Rodents exhibiting one or more of the following signs must be euthanized immediately.)

- Hunched, sleeping posture or recumbence for more than two days
- Unresponsiveness
- Self-mutilation
- Extensive hemorrhage from any orifice
- Lesion or mass that interferes with the normal behavior of the animal (i.e. eating, drinking, locomotion)
- Labored breathing
- Severe weight loss and/or muscle wasting

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18. Monoclonal Production Using the Mouse Ascites Production Model

Animal Care and Use Committee of Wake Forest University

The use of the mouse ascites model for the production of monoclonal antibodies has the potential to cause significant pain and /or distress for animals. Practical *in vitro* methods exist which can replace the ascites method in many experimental applications without compromising the aims of the study. These must be considered before a proposal is submitted. Before proposals that include the mouse ascites method can be approved, the Wake Forest University Animal Care and Use Committee (ACUC) must determine that (1) the proposed use is scientifically justified in the absence of an *in vitro* method, (2) methods that avoid or minimize discomfort, distress, and pain (including *in vitro* methods) have been considered, and (3) the latter have been found unsuitable. Sources of information on *in vitro* alternatives are provided below. When justification for use of the mouse ascites production model is approved by the ACUC, investigators should adhere to the following AGuidelines for Monoclonal Antibody Production Using the Mouse Ascites Production Method.

In-Vitro Alternatives

Investigators are urged to explore *in vitro* alternatives to the use of animals for monoclonal antibody production. Excellent resources include the *Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies*, produced by the Animal Welfare Information Center (AWIC) and the Johns Hopkins School of Public Health Center for Alternatives to Animal Testing (CAAT). In addition, references on alternatives can be found in the document "Institutional Policies and Guidelines on Adjuvants and Antibody Production" (National Academy Press, 1999). Websites for AWIC, CAAT, ILAR and a few other organizations with information on alternatives are provided below.

Animal Welfare Information Center (AWIC)

<http://www.nal.usda.gov/awic/>

Johns Hopkins Center for Alternatives to Animal Testing (CAAT)

<http://caat.jhsph.edu>

Institute for Laboratory Animal Research (ILAR)

<http://dels.nas.edu/ilar/index>

Center for Animal Alternatives (University of California-Davis)

http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm

Public Responsibility in Medicine and Research (PRIM&R)

<http://www.primr.org/>

Guidelines for Monoclonal Antibody Production using the Mouse Ascites Production Method

The following guidelines are summarized from the publication "Institutional Policies and Guidelines on Adjuvants and Antibody Production" [Institute for Laboratory Animal Research (ILAR) Journal, Vol 37, No 3, 1995]. Investigators are encouraged to consult the publication for more detail and other references on the use of, and alternatives to, the ascites production method in mice.

Selection of Animals

Different mouse strains may be used for immunization. As most hybridomas have been derived from the fusion of BALB/c plasmacytomas and BALB/c spleen cells, hybridoma recipient mice must be histocompatible (or immunodeficient/immunosuppressed.) Female

retired breeders are recommended due to their more distensible abdominal musculature and tendency not to fight.

Immunization Protocols

Immunization should be done in accordance with the document "Policy on the Use of Freund's Adjuvant in Laboratory Animals." Primary immunization may be done using Freund's Complete Adjuvant (FCA); subsequent boosters may be done only when Freund's Incomplete Adjuvant (FIA) is used. Intramuscular injections are discouraged in mice. Volumes of 0.05 ml may be injected intradermally. Volumes of 0.05-0.20 ml may be administered subcutaneously. Intraperitoneal (IP) administration is limited to volumes \leq 0.50 ml. Intervals of 14-28 days are recommended between boosts.

Priming Agents

Pristane may be administered at a dose of 0.1-0.5 ml IP, with 0.2 ml/mouse being the dose most often recommended.

Inoculation of Hybridoma Cells

Guidelines for the number of cells in the hybridoma inoculum range from 10^5 to 10^7 cells in basal cell culture media or PBS, inoculated IP in a total volume from 0.1 to 0.5 ml. Hybridomas should be tested for the presence of adventitious agents prior to introduction into an animal host to prevent the potential transmission of infectious agents from contaminated cell lines into mouse colonies.

Abdominal Paracentesis

Guidelines recommend that significant fluid volumes not exceed 20% of the baseline body weight prior to performing abdominal paracentesis. The use of anesthesia for abdominal paracentesis is recommended, although manual restraint is acceptable if done by trained personnel. To reduce bacterial contamination, the paracentesis site should be prepared with an antiseptic. An 18-20 gauge needle may be used to collect ascites fluid. To help prevent shock due to rapid fluid loss, 2-3 ml of warm saline or lactated ringers solution may be administered subcutaneously to the mouse at the time of paracentesis. The number of abdominal taps done in an individual mouse may vary, provided that the procedures outlined in the "Policy on Minimizing Pain/Distress in Rodents" are followed once ascites fluid begins to accumulate.

Clinical Observations

Following paracentesis:

Animals should be monitored for signs of circulatory shock for at least 30 minutes following paracentesis. Signs include hunched posture, inactivity, pallor of the ears and muzzle, tachypnea and dyspnea. Mice with severe or persistent clinical abnormalities warrant consideration for fluid replacement therapy or euthanasia.

Daily:

As stated in the document "Policy on Minimizing Pain/Distress in Rodents," mice used in ascites production procedures must be monitored at least once daily for signs of deterioration. When signs of deterioration are observed, mice must be monitored twice daily until euthanized. Animals showing signs of end-state illness should be euthanized immediately.

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19. Endpoints for Experiments Involving Formation of Neoplasms

Animal Care and Use Committee of Wake Forest University

Death should not be the usual endpoint in studies of neoplasia. The following criteria should be used to determine when to euthanize an animal.

Animals should be euthanized when:

1. the size or location of the neoplasm begins to interfere with the normal behavior of the animal (eating, drinking, locomotion, etc.).
2. the neoplasm develops extensive and/or painful ulceration or necrosis.
3. the signs are observed of end-stage illness as described in the document A Policy on Minimizing Pain/Distress in Rodents. Policy #17.

Trained and experienced personnel must make endpoint determinations. Upon request, training is available from the Animal Resources Program (ARP) veterinary staff. An ARP veterinarian must be consulted if there are questions about the health status of a specific animal. The Wake Forest University Attending Veterinarian has final authority in situations where differences of opinion exist regarding an animal's health status.

REFERENCE: United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia (1998). *British Journal of Cancer* **77**(1), 1-10.

Humane End points and Cancer Research; ILAR 41(2), 2000
http://dels.nas.edu/ilar/jour_online.asp?id=jour_online

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20. Plan for Housing, Exercise and Positive Physical Contact for Dogs

Animal Care and Use Committee of Wake Forest University

Dogs are territorial animals that are adapted for a maximum of locomotor activity in the form of running. It has also been established that dogs are social animals that derive benefit from contact with both other dogs and human beings. With these facts in mind, and in an effort to satisfy the requirements set forth by the United States Department of Agriculture, Wake Forest University has developed the following Plan to provide adequate exercise and socialization for dogs used in research and teaching programs.

Housing and Exercise

In general, dogs will be housed in pens providing at least the minimum required floor space. Dogs will be exercised a minimum of two hours each day if housed in a pen that provides less than twice the minimum floor space required.

Positive Physical Contact

Positive physical contact with human beings will occur daily in all situations of dog housing during routine husbandry and care. If a dog must be housed in a room alone, positive physical contact with people over and above that normally provided during routine care will be provided through physical contact with human beings that encourages exercise by play or similar activities for a minimum of 15 minutes twice a day. A Positive Physical Contact Log Sheet will be posted to document the additional time spent with an isolated dog. The investigator will be responsible for positive interaction in cases when the investigator initiates isolation under individual research protocols. The Animal Resources Program will be responsible for providing dogs with positive physical contact when situations relating to husbandry and health care require individual dogs to be housed in a room alone.

Exemptions

If it is inappropriate for certain dogs to exercise because of their health, condition or well being, the attending veterinarian must document this by completing and signing the form SPECIAL ANIMAL CARE. A copy of the form will be kept with the dog's clinical record. The original will be kept in the Animal Resources Program's office for a minimum of one year and may be kept by the principal investigator thereafter. If a principal investigator determines that it is inappropriate for certain dogs to exercise because of scientific reasons relating to the research protocol, the investigator must provide justification for such an exemption to the Animal Care and Use Committee (ACUC) when the research protocol is submitted for approval. Approval of exemptions will be included as a part of the normal protocol review process. As required by law, exemptions will be reviewed monthly by the attending veterinarian (health exemptions) and annually by the ACUC. (experimental exemptions) Records of any exemptions will be maintained and made available for review for USDA officials or officials of any pertinent Federal funding agency upon request.

7-15-04

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21. Housing of Laboratory Animals

Animal Care and Use Committee of Wake Forest University

I. General Policy

It is the policy of the Medical School that all laboratory animals must be housed in the Central Animal Facilities. This policy is based on the fact that housing in the Central Animal Facilities is necessary for the following reasons:

- In order to assure the animals' well being by providing a controlled environment with regard to temperature and ventilation on a continuous basis.
- To maintain animals in housing areas with ventilation systems separate from areas used strictly by human beings.
- To minimize contamination and odors from animal areas to personnel not ordinarily exposed to animals and any attendant hazards associated with them.
- To meet granting agency and accreditation standards for animal care.

II. Exceptions to General Policy

The only exceptions to housing animals in the Central Animal Facilities are made on the basis of scientific requirements of particular research projects. Decisions concerning whether there is a scientific basis for housing animals other than in the Central Animal Facilities can be made only by the Animal Care and Use Committee. Requests for exceptions presented to the Animal Care and Use Committee must describe how the Animal Care Standards of the Bowman Gray School of Medicine, including the care requirements of the American Association for Accreditation of Laboratory Animal Care as outlined in the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, 1996) will be met.

Requests to the Animal Care and Use Committee for exceptions to the General Policy must address specifically each of the following points:

- Location and characteristics of housing facility.
- Ventilation and temperature control.
- Lighting.
- Cages – size and construction.
- Sanitation practices with regard to facilities, cages and other equipment.
- Waste disposal.
- Vermin control.
- Provision of food and water.
- Identification of animals and animal records.
- Daily observation and care of animals, including weekends and holidays.

III. Prior Approval for Exceptions

Before grant applications or contract proposals that require an exception being made to the General Policy, there must be two stages of prior approval:

- First, the Animal Care and Use Committee must assure that a scientific reason exists for an exception being made to the General Policy and that their files include an assurance from the Principal Investigator that all of the items listed above have been addressed specifically.
- Second, if renovations will be required in order to make an exception to the General Policy, an understanding must be reached between the Administration and the Principal Investigators relevant to sources of funds for such renovation costs before the grant or contract is submitted.

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22. TRANSPORTING ANIMALS

Transporting living animals through the medical center Animal Care and Use Committee of Wake Forest University

I. SCOPE:

Live animals are routinely transported through research and nonresearch areas of the Hawthorne Campus. This transportation consists of moving animals from the vivaria to and from laboratories and other facilities within the institution (e.g. MRI building). All transport of animals among WFU campuses and between WFU and other institutions must be coordinated through the ARP. Under most circumstances the ARP will conduct or arrange the transportation. The ARP and the WFU ACUC must approve exceptions.

Inherent to this transport of live animals are several risks and concerns of which investigators and all personnel working with animals need to be aware. These concerns include ones of security, environmental health and public relations. It is important to minimize these risks to both the animals and the public who come in contact with them during their transport.

II. PURPOSE:

The purpose of this policy is to identify appropriate methods of transporting live animals through the institution to reduce the inherent risks associated with this transport.

NOTE: This policy does not address sheep. Sheep must not be transported through the Medical Center because of the potential aerosol transmission of Q fever during their transport (even with proper draping of the animal).

III. PROCEDURE:

A. Manner of Transport

1. Containers – Animals must be transported in self-contained receptacles that prevent contamination of corridors and elevators from secretions and excretions (blood, fluids, urine and feces) and prohibit escape of the animal. In addition, appropriate steps must be taken to avoid visible blood on carts, transport cages or equipment during transport. Personnel involved in transporting animals are responsible for cleanup of animal body fluids or wastes that accidentally contaminate corridors or elevators.

2. Visualization – Transportation must occur in a manner that prevents direct visualization of the animal. This may be accomplished by completely draping an anesthetized animal, draping the transport cage or transporting the animal in an opaque ventilated container.

3. Protective Clothing – Laboratory coats and gloves should be worn during transportation of animals. Species-specific full protective clothing must be worn before performing procedures upon animals in the laboratory or patient service areas (e.g., MRI, PET, CAT).

4. Elevator Use –

a. Animals must not be transported in elevators that are carrying nonMedical Center personnel. If an investigator is using an empty elevator to transport the animal, request that nonMedical Center personnel attempting to enter the elevator wait for the next available elevator.

b. A service elevator in the Nutrition Center is available for the transporting of animals. This elevator will not be available for public use, thereby reducing the interaction between animals and the public. Investigators should use this elevator to transport animals.

5. Transport Routes –

For transporting animals to the MRI/PET Building or the CAT scanner, animals must not be transported along the ground floor corridors of Watlington Hall, the West Building, North Tower, or the Reynolds Tower. These corridors are high public traffic areas. The only approved routes to these diagnostic areas are described in the attached pages.

6.Route Approval –

a. For transportation of animals to patient service areas other than MRI, PET or CAT, approval of the precise route of transport of animals through the hospital or any patient service area must be received from the North Carolina Baptist Hospital Infection Control Committee Chair (or their designee) prior to any animal transport.

b. Proposed transportation routes may be directly submitted to the Director of the Bowman Gray School of Medicine Environmental Health and Safety or the Director of the North Carolina Baptist Hospital Infection Control.

c. Transportation of animals through the hospital that are injected with radioactive substances must be approved by the Radiation Safety Officer in Environmental Health and Safety Office.

7.Between Campus Transport – Transfer of animals between campuses (PTCRC, Reynolds, Hawthorne or Friedberg) is the responsibility of the staff of the Animal Resources Program or the Laboratory Animal Medicine Core of the Friedberg Campus.

8.Animal Escape – The Animal Resources Program and Security should be notified immediately in the event that an animal escapes during transportation and can not be recaptured.

B.Approved Routes to Transport Animals To MRI/PET and CAT

1.MRI/PET Building –Animals should be transported from the vivaria to floors 2-5 of the Gray Building and into the main north-south corridor of floors 2-5 in Watlington Hall. Passing the main bank of elevators, follow the corridor that bears to the left down the center of the building (the right corridor will lead to patient care areas in the West Building) to the elevator that is about three-fourths of the way down the corridor in Watlington Hall. Take this elevator to the first floor and continue into the corridor that leads to the bridge to Meads Hall. This first floor corridor leading to the bridge is almost directly across from the elevator. Keep to the right while traveling in this corridor to continue into the bridge leading to Meads Hall. Immediately after the bridge, take the elevator on the right to the G floor and follow the main corridor of Meads Hall to the north-south spine of the hospital (near Employee Health). Turn left at the spine and follow the tunnel under Queen Street to the PET Center on that level or take the elevator (which should not contain any nonMedical Center personnel) to the first floor of the MRI Building to access the MRI facility. Before exiting the elevator on the first floor, determine that patients or visitors are not in the main lobby of the MRI Building. Request that nonhospital personnel move temporarily to the MRI waiting area before transporting the animal from the elevator to the MRI holding room.

2.CAT Scanner –

Animals should be transported from the vivaria to floors 2-5 of the Gray Building and into the main north-south corridor of floors 2-5 in Watlington Hall. Passing the main bank of elevators, follow the corridor that bears to the left (the right corridor will lead to patient care areas in the West Building) to the elevator that is about three-fourths of the way down the corridor in Watlington Hall. Take this elevator to the first floor and continue into the corridor that leads to the bridge to Meads Hall. This first floor corridor leading to the bridge is almost directly across from the elevator. Keep to the right while traveling in this corridor to continue towards the bridge leading to Meads Hall. Immediately before the bridge, turn right into the corridor leading to X-ray. Follow the corridor and make a left into the intersecting corridor. As you pass the elevators in this corridor you will be entering the

Cardiac Catheterization/Special Procedures areas of Radiology. Continue down the corridor and make a right into the second corridor on your right. This corridor will lead directly into the CAT scanning area.

IV. Animal Care and Use Committee

The route and method of transportation of animals through the institution for experiments that will be conducted in the MRI/PET Building, CAT scanner or any other patient service area should be described in the Animal Care and Use Committee protocol that is submitted for the study.

V. Additional Questions or Special Needs:

Additional questions or special requests/needs should be addressed to the Bowman Gray School of Medicine Environmental Health and Safety Office (716-9375, beeper 773-9076) or the North Carolina Baptist Hospital Infection Control Office (716-3482; beeper, 773-9814, after hours and holidays, beeper, 773-8001).

Transport of Monkeys

When surgery, necropsy or a PET procedure must be done, monkeys will be sedated and transported to the corresponding suite in accordance with the Wake Forest University Animal Care and Use Committee's policy. Transporting living animals throughout the Hawthorne campus of the Medical Center. The proposed routes are as follows:

For Transport of Monkeys from the 5th floor of the Nutrition Research Center (NRC) building housing area to the 6th floor NRS surgery and necropsy suites:

A. Exit the north door of the 5th floor NRC building facility and take the north stairwell to the 6th floor NRC building. Access the 6th floor animal facility and proceed to the survival surgery or necropsy suite (See figures 1 and 2).

B. Exit the south door of the 5th floor NRC building facility and take the service elevator (or south stairwell) to the 6th floor NRC building. Access the 6th floor animal facility and proceed to the survival surgery or necropsy suite (See Figures 1&2)

For Transport of Monkeys from the 5th Floor of the NRC building to the PET Center (MRI Building):

Exit the south door of the 5th floor of the NRC building facility and take the service elevator (or south stairwell) to the 4th floor of the NRC building. After exiting the service elevator, turn right and access the 5th floor Hanes building via the connecting hallway. [Note: The 4th floor of the NRC building is on the same level as the 5th floor of the Hanes building.] Proceed to the 5th floor of the Gray building via the connecting walkway and follow the approved route from this point to the PET Center as outlined in the policy on animal transport.

Reviewed 9/02

23. Policy on Monitoring Compliance with Approved Protocols

Animal Care and Use Committee of Wake Forest University

1. During the period of approval of each protocol, an annual review will be conducted by exchange of memoranda to confirm that no changes have taken place in the approved activity, which might require further consideration by the ACUC.

2. All investigators will be notified by the ACUC of any new requirements of PHS, USDA, or the institution.

3. Two mechanisms will be used to assess compliance:

A: On the anniversary of approval of a protocol, a standard form giving basic ACUC information, e.g. approval number, date, title, species, etc., will be sent to the Principal Investigator. The investigator will note that either no changes have taken place, or the P.I. will describe any changes that have occurred.

The ACUC staff will separate the responses, reviewing and filing those that indicate no changes and forwarding the remainder (change indicated) to an ACUC member designee for assessment of the changes reported. After review or inquiry by the ACUC designee, any changes to the approved activity which are deemed of sufficient magnitude to merit further consideration will then be presented to the Chairman of the ACUC or the full ACUC.

B: Immediately prior to each semi-annual ACUC facility inspection, a memorandum will be sent to each investigator with one or more approved ACUC protocols. During each semi-annual inspection, all active protocols (those actively utilizing animals) will be noted. From the pool of active protocols, the Program Evaluation Subcommittee will select four protocols at random, two from the Bowman Gray Campus, one from the Friedberg Campus, and one from the Reynolda Campus/Chestnut Campus/other facilities. Each subcommittee will visit the corresponding Principal Investigator, with the Program Evaluation subcommittee reviewing the second protocol from the Bowman Gray Campus. Each subcommittee will schedule a visit with the Principal Investigator and review the protocol with the P.I. and appropriate personnel. During the visit with the P.I., each subcommittee will consist of two or more scientist members of the ACUC plus a non-scientist whenever possible. The subcommittee is responsible for submitting a written summary of their visit as part of the facilities inspection or program evaluation written report. If any issue(s) of non-compliance are found that are deemed serious, such as activities that are not described in the protocol, the subcommittee will notify the Chairman of the ACUC to initiate action to correct the issue(s).

4. Protocols identified through the *Animal Concern Hotline* as having a problem or concern will also be investigated for compliance with the description of procedures in the protocol. Additionally, any member of the ACUC may suggest additional protocols to be reviewed in this manner.

4. All of these dispositions are to be documented as official ACUC actions.

Adopted June 17, 1997

re-issued 12/98
reviewed 11/16/04

24. The Wake Forest University Nonhuman-Primate Environmental-Enhancement Plan

Animal Care and Use Committee of Wake Forest University

I. Statements of Goals

A. Goals:

Wake Forest University supports the use of nonhuman primates in biomedical research, breeding, and education as deemed appropriate to the mission of the institution and approved by the ACUC. These activities may require animals to be maintained in conditions different from those that would be experienced in nature. Nevertheless, it has been our long-established policy to employ all reasonable means to ensure the psychological well-being of nonhuman primates while not compromising the scientific objectives of research. In support of this policy, the following Plan for Psychological Well-Being of Nonhuman Primates (the Plan) has been developed and is designed to conform to current Federal regulations regarding environmental enhancement and psychological well-being.

In designing the Plan, we recognize that the best procedures will be based on information that, while incompletely developed, will vary as to species, age, gender and physical condition of the nonhuman primates. Together with the emerging literature on psychological well-being of nonhuman primates, we will rely considerably on the experience of our own investigators who have worked with a variety of species in captive and naturalistic environments. Our ultimate goal is to implement the best procedures available to provide for the psychological well-being of captive nonhuman primates. A subcommittee of the ACUC will provide oversight of these activities. The application of the Plan will be reviewed on a case-by-case basis for each research protocol submitted to the ACUC. A primary emphasis for performance criteria rather than engineering standards will be used to judge individual Plans.

This document is intended to provide the background and overall principles by which the methods for psychological well-being are to be administered. The Plan also describes the methods by which these goals are to be attained. These methods are based on best-practice guidelines derived from the existing literature and from the experience of faculty trained in primatology. The situations addressed are those encompassing the types of experiments at WFU that use nonhuman primates. Furthermore, the Plan specifically addresses species currently housed at WFU. As experimental paradigms evolve to include new species or housing conditions, the Plan will be revised accordingly.

To be in compliance with the Plan, an experimental protocol involving nonhuman primates must include a study-specific standard operating procedure (SOP) describing the strategies used to achieve environmental enrichment. Furthermore, the study-specific SOP must justify any deviations from the enrichment methods suggested in the Plan. These study-specific SOPs will be submitted at the same time and become part of the approved ACUC protocol. They will be reviewed and renewed annually, and will expire with the protocol after three years. These study-specific SOPs will also be

provided as guidelines to personnel working on the protocols, and will be readily available for inspection.

B. The aims of this plan include:

- provision of a housing environment that allows for the expression of a broad range of species-typical behaviors, including locomotion, social interactions (if consistent with experimental design), foraging, and manipulation.
- minimization of potentially injurious aggression and self-injurious behavior through provision of maximal environmental enrichment consistent with experimental constraints.
- establishment of a program to insure that proven enrichment techniques are applied to all nonhuman primates in a manner consistent with experimental constraints. Depending on the species and the experimental constraints, strategies may include the provision of manipulable objects, foraging situations or apparatuses, and furniture for climbing, resting, perching, or locomoting above the floor.

Federal guidelines and Wake Forest University Policy require that all individuals working with nonhuman primates be required to receive training on NHP environmental enrichment methods. Education and training of personnel is of paramount importance to the successful implementation of the Plan. All individuals working with NHP are required to receive training with regard to natural history, behavior, husbandry and enrichment of the species with which they are working. This information is described in this Plan.

II. Pertinent Information

A. Natural History

1. **Overview.** The majority of nonhuman primates maintained for research at the Wake Forest University fall into two genera: 1) *Macaca* (macaques, including rhesus and cynomolgus monkeys); and 2) *Cercopithecus* (guenons, including the widely distributed vervet or African green monkey). The Institution also maintains a small number of squirrel monkeys (genus *Saimiri*). Macaques and vervets are collectively referred to as Old World monkeys, reflecting their geographic origin. In contrast, squirrel monkeys are New World monkeys. The relevant natural history of these groups is described below.

2. **Macaques and Vervets.** These animals can be categorized as highly social omnivores. Their social groups are organized along matriline, vary greatly in size depending on the environment (*C. aethiops* group size=7-76, average=12-24; Bloomstrand and Maple 1987; *M. fascicularis* 10-70; average=18-48 Melnick and Pearl, 1987), and typically include more than one male. Males leave the natal group at around the time of puberty and may live alone or in all male bands for a time. Males emigrate into new groups, and may change social groups over

their lifetime. All species are generally considered terrestrial, however *M. fascicularis* are notable for their arboreality relative to the other species.

Despite this broad similarity, the taxa differ in aspects of social organization and behavior which may influence the way they respond to captive environments. Vervets appear to be more territorial than macaques (Fedigan 1982), and within a social group, vervets appear to maintain greater interindividual distances than macaques. Macaque aggression in captivity tends to be more asymmetrical and unidirectional, more ritualized, and less often related to wounding or killing than vervet aggression in captivity. Vervets use space to reduce aggression, whereas macaques depend more on ritualized social communication to reduce aggression. Thus, macaques may accommodate higher social densities than vervets. These characteristics should be considered when developing captive management programs. Different strategies may be needed to manage these two kinds of monkeys in captivity. On balance, the literature suggests that vervets may require more ways to hide and escape when housed socially than macaques.

3. **Squirrel monkeys.** Like all other New World monkeys, squirrel monkeys are arboreal. Squirrel monkeys typically forage on fruit and insects, often flushing out the latter by thrashing through the canopy or leaf litter. In the South American forests of their origin, squirrel monkeys typically live in multimale-multifemale matrilineal groups of 10 to 35 animals. There is typically a cohesive female social core that accounts for most of the affiliative and agonistic interactions within the group. During the nonmating season, adult males travel with the group but tend to remain spatially distant from females and other males. During the mating season, however, adult males become actively engaged in relations with other animals, especially in dominance hierarchy alignments and aggression (Robinson and Janson, 1987). Furthermore, there are species, and possibly subspecies, differences in social structure among squirrel monkeys (e.g. social status, emigration, kinship patterns within groups etc. (Rowe, 1996). The management of squirrel monkeys used in research should take the foregoing ecological and behavioral characteristics into consideration with regard to decisions about cage design and social group structure. In particular, effort should be made to provide elevated perches and to provide housing in separate rooms/areas for males and females.

B. Records

Records documenting the source of the animal, its rearing history, and its housing history will be maintained if available. ARP will maintain health records for each individual nonhuman primate. Documentation of an animal's environmental enrichment history shall consist of the ACUC protocol and accompanying study-specific SOP under which the animal is enrolled and which contains details of the environmental enrichment plan for animals in the study. In addition, an entry will be made in the daily room/building log noting the observation of injurious behavior such as self-wounding. When an observation of self-injurious behavior is noted, the observer should contact the Environmental Enrichment Coordinator and Principal Investigator. The Environmental Enrichment Coordinator will

arrange for an assessment of the animal and, if advised, will consult with a recognized expert in nonhuman primate behavior. If possible, a plan for remediation will be formulated with the research and veterinary staff and presented to the Principal Investigator. It will be the responsibility of the Principal Investigator to insure that the agreed-upon plan for remediation is implemented. Attempts at remediation and response to such efforts will be documented in the animal's record. It is recognized that there may be no known methods of remediation for some injurious behaviors and, further, that for some individual animals all known methods of remediation might prove ineffective. In the latter case, the Attending Veterinarian in concert with the Principal Investigator will determine the final disposition of the animal.

III. General Considerations regarding Social Housing and Implementation of the Plan.

This plan provides for the social housing of each animal. Exceptions to the policy that each animal shall be socially-housed are detailed in Section V.

- A.** The position of WFU is that all animals shall be socially housed, either paired or grouped, unless contraindicated by individual history (Part II, above) or exempted under Section V, "Special Considerations".
- B.** Animals housed singly generally benefit from visual, auditory, and olfactory contact with nearby conspecifics. Unless contraindicated by individual history or exempted under Section V and approved by the IACUC, all single-caged animals will be located to maximize the beneficial aspects of this sensory stimulation. Housing may include a transparent wall, or a wire mesh wall that promotes some social experience while insuring the safety of the animals.
- C.** Professional judgments regarding study-specific enrichment protocols will be provided by the Environmental Enrichment Coordinator, the NHPEE committee, or the Institution's recognized experts in primatology.

IV. Environmental Enrichment

A. Pair- and Group-Housed Monkeys

Macaques, vervets, and squirrel monkeys are gregarious species that spend most of their lives in social groups. As such, it is reasonable to assume that pair- and group-housing provides an important form of environmental enrichment. There are other forms of enrichment that may also be made available to socially-housed monkeys as based on professional judgment and published evidence. These are described below.

- Structural enrichment. This includes perches and other cage structures suitable for climbing, sitting, standing, and generally engaging in the range of species-typical locomotor and postural activities. In addition to perches, the bars of a social pen may be considered to provide an adequate substrate for locomotion.

Three-dimensional opportunities for leaping and staying off the floor must be provided for squirrel monkeys. All social pens and corrals should be provided with structural enrichment sufficient for the number of animals housed therein.

- Provision for Flight. Animals should be provided with the means of temporarily escaping from the view of other animals. Space itself may be an adequate barrier for animals. Open plastic barrels can also be used to provide temporary respite.
- Object Enrichment. This includes durable manipulable objects such as mirrors, plastic balls, cone-shaped rubber toys, and gnawing sticks that are safe to be used by monkeys.
- Food Enrichment. This includes puzzle boards and other food enrichment devices that are safe to be used by monkeys. Food enrichment also includes the provision of fruits and vegetables, especially those that require manipulation or extraction for consumption. Food enrichment should be provided for all nonhuman primate unless prohibited by experimental constraints
- Sensory and Occupational Enrichment. In some cases the monkeys may participate in daily experimental sessions. This may involve manipulating joysticks, response levers, or making operant responses to obtain food pellets. After initial training, it is clear that such rewarded responses contribute to psychological enrichment. These experimental situations are recognized as part of the entire enrichment program

B. Single-Housed Monkeys

Species-typical behavior may make it more difficult to house some species socially than others. Likewise, the individual behavioral characteristics of some animals may prohibit safe social housing. All monkeys excluded from social housing require an exemption in accordance with Section V, "Special Considerations." This request for exemption will be reviewed by the ACUC. Individually-housed nonhuman primates should, whenever it is possible, have visual, auditory, or olfactory contact with each other. In addition, other forms of enrichment, i.e., enhanced structure/substrate, manipulanda, and foraging opportunities, may take on an increased importance. Use of food treats and presentation of food can be part of the overall enrichment program. Development of the enrichment program should take into account species-typical behavior, individual personality differences and preferences, and the provision for novelty in its application.

Individually-housed animals that may require additional or enhanced attention include: infants and juveniles with regard to their physical and social development; socially-isolated monkeys; monkeys that exhibit self-injurious behavior; and animals whose activity is restricted for longer than 12 hours for experimental purposes.

Cage enrichments will be provided for individually-housed primates. Exemptions for scientific reasons will be considered in accordance with Section V, "Special

Considerations”, and with 9 CFR “Animal Welfare”; “Part 3-Standards”; §3.81. Enrichment devices should be carefully selected on the basis of the behavior of the species that is being provided opportunity for expression of species-typical behavior. For the sake of simplicity, these devices are placed into one of three categories: structural enrichment, object enrichment, and food enrichment. These enrichment approaches should be used on an individual-response basis and based on discussions with, and observation by, the research staff and Environmental Enrichment Coordinator. Furthermore, some psychological enrichment is derived from the experimental tasks, as discussed below. Examples include:

- **Structural Enrichment.** This includes perches and other cage structures suitable for climbing, sitting, standing, and generally engaging in the range of locomotor and postural activities typical for the species. Three-dimensional opportunities for leaping and staying off the floor must be provided for squirrel monkeys. Perching should be as extensive as possible within the inherent space limitations of single cages.
- **Object Enrichment.** This includes durable manipulable objects such as plastic balls and cone shaped rubber toys specifically manufactured for use by monkeys. Gnawing sticks and other non-nutritive manipulanda can also be employed. Such objects should be provided to all animals and rotated on a regular basis to insure novelty.
- **Food Enrichment.** This includes but is not limited to puzzle boards and other food enrichment devices that are safe to be used by monkeys. Food enrichment may also include the provision of fruits and vegetables, especially those requiring manipulation or extraction. Ice cubes or other non-nutritive objects can also be used for enrichment. Food enrichment should be provided to all animals unless prohibited by experimental constraints.
- **Sensory and Occupational Enrichment.** In some cases, the monkeys may participate in daily experimental sessions. This may involve manipulating joysticks, response levers, or making operant responses to obtain food pellets. After initial training, it is clear that such rewarded responses contribute to psychological enrichment. However, the experimental situations will not constitute all, but are recognized as part of, the entire enrichment program for singly housed animals.

V. Special Considerations

All nonhuman primates are included in the Plan unless excluded for health or well-being reasons, or for other cause and approved by the ACUC upon recommendation by the NHPEE Subcommittee. Further, any animals that are not housed in social situations require an exemption, and that exemption must be documented.

In the event of illness, an exemption terminates when the animal finishes treatment. Long-term exemption from social housing may be authorized by the attending veterinarian or her/his designee under the following conditions:

1. Permanent clinical debilitation due to extreme injury or old age
2. Contagious disease
3. Aggression
4. Social incompatibility
5. First 31-45 days of quarantine or until released from CDC quarantine
6. Other

VI. Monitoring

A. Records

Each animal is observed daily by a variety of staff to assess signs that reflect the animal's state of health, i.e., activity, appetite, stool and urine production, hydration, injury, the presence of self-injurious behavior, etc.. Deviations from an animal's normal behavior documented by appropriately trained personnel will be recorded in the ARP log (i.e., room observation form) and should be reviewed by veterinary and research personnel. If determined appropriate (i.e., no physical cause for the deviation in state can be determined), then the observation should also be recorded in the enrichment log.

B. Remediation

An appropriately trained person, such as experienced research or animal care staff, the Environmental Enrichment Coordinator, or a veterinarian, should further investigate any injurious behavior. A provisional diagnosis and strategies for intervention should be recorded by veterinary staff in the animal's clinical record and by research staff in the enrichment log. Both successful and unsuccessful remediation strategies are documented. It is recognized that there may be no known methods for remediation of some physical impairments and abnormal behavior. Further, that for some individual animals, all known methods of remediation might prove ineffective.

C. Assessment of the Plan

Compliance with the plan is deemed successful if regular monitoring reveals that an approved, experiment-specific enrichment plan is applied to all animals and that injurious behaviors are noted and acted upon as described in the previous sections of this document.

This document will be reviewed by the WFU ACUC, and its subcommittee on Nonhuman Primate Environmental Enrichment, no less frequently than every three years.

Reviewed 07/2004
Reviewed 10/16 /2004
Approved by NHPEE 11/8/04

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Approved 11/16/04

25. Adoption Policy

Animal Care and Use Committee of Wake Forest University

This policy describes the conditions and procedures for the release of living research animals to private individuals for use as pets or livestock. The ARP Director will oversee the adoption process and can authorize the release of any animal if this action is judged not to be contrary to the best interest of either the animal or Wake Forest University (WFU). The principles embodied by this policy are that: (1) the adoption of an animal as a pet, or the release of an animal as livestock, can be an appropriate alternative to euthanasia; (2) the policy is designed primarily to be used by individuals associated with WFU; (3) the release of any animal carries the risk of potential liabilities to the adoptive owner, WFU and the community.

It is further recognized that some species of research animals may not be appropriate for adoption; these include, but are not limited to, non-human primates and bats. The decision as to any species suitability for adoption resides with the ARP Director. Animals will not be released for adoption to individuals who intend to subsequently utilize them as food. Dead animals, or parts of dead animals, owned by WFU may not be removed or used for private purposes under any circumstances.

The following conditions must be satisfied before any research animal will be released.

1. An animal should not be considered for adoption until it has been utilized for the intended research or educational purposes for which it was acquired.
2. Although designed primarily to facilitate in-house adoptions, in certain instances, and at the discretion of the ARP Director, custody may be granted to an individual for the expressed purpose of subsequently transferring custody of the animal to a rescue league or humane society.
3. The prospective adoptive owner, an adult, shall demonstrate to the ARP Director, or to the Directors representative, the capacity to provide for the needs and physical and emotional well-being of the animal.
4. The prospective adoptee will be examined by ARP veterinary personnel and determined to be sound and clinically healthy; it must have a normal life expectancy at the time of release. The animal must neither be disfigured nor shall any outward signs of prior invasive procedures be present. The animal must not have been exposed to any experimental infectious agents or hazardous compounds. The animal must have a temperament appropriate for the species. The animal will have received all appropriate vaccinations required for housing at WFU. However, animals are not guaranteed to remain healthy or to be housebroken (as applicable). In addition, because any individual animal may be an asymptomatic carrier of diseases transmissible to humans, WFU makes no claims that the animal is not infected at the time of release.
5. The examining veterinarian must sign the WFU Animal Adoption Release Form indicating that he/she has examined the animal and answered any questions the adoptive owner had about the animals experimental use, health, and/or care.
6. The principal investigator must sign the WFU Animal Adoption Release Form indicating approval of the adoption and release of the animal.
7. The prospective adoptive owner must sign the Animal Adoption Release Form indicating that he/she understands and agrees to the conditions of the adoption and releases WFU and its employees from all claims or damage to property or persons caused by the adopted animal.

8. By signing the Animal Adoption Release Form, the adoptive owner acknowledges that:

a. He/she is adopting the animal solely as a personal pet and not for the purpose of transferring the animal to a third party or for use in any illegal or harmful activity that may have adverse effects upon the animal, WFU or the community.

b. He/she understands that an examination of the animal has been conducted by an ARP veterinarian, and he/she has had the opportunity to ask the examining veterinarian health- and care-related questions.

c. He/she has inspected the animal personally and found it to be in good physical condition and suitable as a pet. He/she understands that there is no guarantee that the animal will remain healthy or is housebroken (as applicable).

d. He/she is fully aware that the animal has been the subject of a research study at WFU, and he/she has had the opportunity to discuss the specifics of the research with the examining veterinarian.

e. He/she agrees to accept full responsibility for the animal, including any and all future costs related to the animals care and well being. He/she agrees to comply with all applicable laws and ordinances for the municipality in which the animal is kept and understands that WFU strongly recommends spaying or neutering the animal if it is a dog or cat.

f. He/she assumes the risks related to adoption of this animal and releases WFU and its employees from all claims for damage to property or persons caused by the animal.

g. He/she understands the conditions of adoption, agrees that all terms are fully understood, and voluntarily accepts a full and final adoption of the animal.

An Animal Adoption Release Form can be found in Appendix VII.

Approved 8/03 revised 12/16/04

26. Use of Expired Medical Materials and Non-Pharmaceutical-Grade Compounds in Research Animal Care and Use Committee of Wake Forest University

Expired Medical Materials

The use of expired medical materials such as drugs, fluids, or sutures on animals is not considered to be acceptable veterinary practice and does not constitute adequate veterinary care as required by the *Guide for the Care and Use of Laboratory Animals* and regulations promulgated under the Animal Welfare Act. Consequently, the use of expired medical materials in animals used in teaching and/or research at Wake Forest University is prohibited except for the following circumstance:

The use of expired medical materials in acute terminal procedures is allowed, as long as their use does not adversely affect the animal's well-being or compromise the validity of the scientific study.

Note: Anesthetics, drugs administered to relieve pain or distress, emergency drugs, and euthanasia agents must not be used beyond their expiration date, even when used in acute terminal procedures.

All expired medical materials maintained for use in terminal procedures must be segregated in an appropriately labeled, physically separate location from non-expired medical materials. Each individual expired item should be clearly marked so as to avoid unintentional use of expired materials.

Non-Pharmaceutical-Grade Compounds

Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute, terminal procedures. Non-pharmaceutical-grade chemical compounds should only be used in animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings alone are not an adequate justification for using non-pharmaceutical-grade compounds in animals.

8/24/01
Reviewed 12/16/04

27 . Food and/or water "regulated" animals

Animal Care and Use Committee of Wake Forest University

Animals performing an experimental task may require control of diet or fluid intake to shape behavior. In addressing the care and use of such animals, the principal investigator (PI) must satisfy the requirements of both the United States Department of Agriculture (USDA) and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) with oversight from the Animal Care and Use Committee (ACUC). The USDA describes procedures that involve “food or water deprivation beyond that necessary for normal presurgical preparation” as causing more than momentary or slight distress. Under the USDA policy for Painful/Distressful Procedures (Policy #11)¹, animals exhibiting signs of distress are expected to receive appropriate relief unless written scientific justification is provided in the ACUC protocol and approved by the Committee.

Relevant excerpts from the National Research Council’s (NRC) “Guide for the Care and Use of Laboratory Animals²” are presented in the following paragraph.

“When experimental situations require food or fluid restriction, at least minimal quantities of food and fluid should be available to provide for development of young animals and to maintain long-term well-being of all animals. Restriction for research purposes should be scientifically justified, and a program should be established to monitor physiologic or behavioral indexes, including criteria (such as weight loss or state of hydration) for temporary or permanent removal of an animal from the experimental protocol (Van Shuyters and Oberdorfer 1991). Restriction is typically measured as a percentage of the ad libitum or normal daily intake or as percentage change in an animal's body weight.

Precautions that should be used in cases of fluid restriction to avoid acute or chronic dehydration include daily recording of fluid intake and recording of body weight at least once a week (NIH 1990)-or more often, as might be needed for small animals, such as rodents...”

In order to comply with the requirements written above, the Wake Forest University ACUC has established the following policy to cover experiments that involve food or fluid regulation of research animals.

- Food and fluids may be restricted beyond that necessary for normal presurgical preparation only when written scientific justification is provided in the ACUC protocol and approved by the Committee. Please note that a concise, detailed justification will help the Committee evaluate the need for food or fluid regulation and shorten the approval process.
- The PI shall describe in the ACUC protocol what method(s) will be used to determine normal body weight (BW).
- All animals that are food or fluid regulated must be observed daily by the PI’s staff conducting the restriction. In addition, animals housed in Animal Resources Program (ARP) facilities must be observed daily by the ARP animal care and /or veterinary staff.
- When regulating food, the PI must record body weights at least weekly for animals weighing > 1 kg and daily for smaller animals weighing ≤ 1 kg. When an animal loses more than 25% of its “normal BW” (as defined in the ACUC protocol), the PI’s staff must contact the ARP veterinary staff immediately to assess the condition of the animal. The veterinary staff and the PI will work together to address the weight loss.

In the event of a difference of opinion, the Attending Veterinarian will make the final decision as to the course of action to be taken.

- When regulating fluid intake, the PI must record experimental and supplemental fluid intake in order to evaluate total daily fluid intake. If the Attending Veterinarian or designee determines that an animal is dehydrated on the basis of either physical examination or clinical pathologic measurements or if the animal's BW falls below the 25% limit, the laboratory must contact the ARP veterinary staff immediately to assess the condition of the animal. The veterinary staff and the PI will work together to address the weight loss and/or dehydration. In the event of a difference of opinion, the Attending Veterinarian will make the final decision as to the course of action to be taken.

- Exceptions to the 25% BW limit are allowed only if the Attending Veterinarian determines that the animal is healthy and not compromised or if prior approval has been obtained from the ACUC for the **specific study proposal**.

- The attached charts or similar records providing the same information are required for recording BW and/or fluid intakes.

- The charts must be posted in proximity to the animals and readily available at all times to all personnel (laboratory, ARP, or ACUC).

- As a chart becomes full, the PI must retain the documents until the completion of the study and have these documents readily available for inspection.

Appendix V: Body Weight Chart for Food Regulated Animals

Appendix VI: Body Weight/Fluid Intake Chart for Fluid Regulated Animals

¹ <http://www.aphis.usda.gov/ac/policy/policy11.html>

² <http://www.nap.edu/readingroom/books/labrats/>

28. Overcrowded Mouse Cages

Animal Care and Use Committee of Wake Forest University

I. Purpose

This policy is designed to ensure that mice are housed in a manner that meets Federal guidelines for adequate floor space per mouse. Specifically, the Guide for the Care and Use of Laboratory Animals lists the following floor space requirements for mice.

<u>Weight, g</u>	<u>Floor Area/Mouse, in²</u>
<10	6
10-15	8
15-25	12
>25	15

This policy was written in an attempt to facilitate the day-to-day application of these requirements in WFU animal facilities. Investigators are required to have their staff closely monitor their breeding colonies and wean the offspring at 25 days of age to avoid overcrowded cage notification.

II Standard Densities for a 75 sq. inch mouse cage

- 5 mice of weaning age or older per cage
- No more than 1 litter per cage
- No more than 2 adults and 1 litter per cage

III. Violations of Standards

A. Investigators are given 48 hours from the time of the notification by the ARP to resolve the overcrowded cage(s). **An investigator is in violation of cage density standards at the time of notification**, not upon failure to correct the overcrowded condition.

B. After the third notification within six months for a specific investigator, the ARP will automatically separate overcrowded cages upon initial discovery and the IACUC will be notified. If no violations occur within a subsequent six-month period, the ARP will revert back to the three notification procedure before automatically separating the cages.

C. It is imperative that the correct Date-of-Birth (DOB) for each litter be clearly identified on the cage card. In addition, once a litter has been weaned, the DOB must be crossed out or the female cage card replaced. Weaning typically is done at 21 days of age. However, some strains produce very small offspring and these mice may have difficulty accessing food and water. Therefore, pups up to 25 days of age may be kept with the mother.

D. Exemptions or extensions to the three caging condition listed in Section III. A. must be approved by the IACUC. An "Overcrowded Mouse Cage Exemption/Extension Request Form" (Appendix X) is available through the ARP Operations Coordinator or the ARP office. This form should be completed and submitted to the ARP Operations Coordinator. The ARP veterinary staff will provide a recommendation to the IACUC based on a review of the request. Requests will then

be reviewed by the IACUC. The IACUC will then notify the PI and the ARP of their decision and any time limitations established.

IV. ADDITIONAL QUESTION OR SPECIAL NEEDS
Overcrowded Mouse Cage Exemption/Extension Request Form (Appendix X)

Prepared:

Approved: 5-14-04
Reviewed 12/16/04

29. Surgical Procedures in Rodents

The purpose of this document is to outline procedures for aseptic surgery and post-operative care of rodents. Aseptic technique is used to reduce microbial contamination to the lowest possible practical level. Use of anesthetic and analgesic agents to minimize any pain associated with the experimental procedure is scientifically and ethically imperative. All personnel involved in rodent surgery should read this document and understand what the requirements for aseptic technique and appropriate peri- and post-operative care.

Major surgeries include invasion of the cranial, sinus, abdominal or thoracic, cavities. Any procedure that might leave the rodent with a permanent handicap, either physical or physiological, would also be considered major surgery (i.e. amputation of a limb).

Minor surgeries include those that do not expose a body cavity and cause little or no physical impairment. An example of a minor survival surgery would be placement of subcutaneous implant.

The number and size of subcutaneous implants should be the lowest number and smallest size as possible. The subcutaneous implants must not impede normal mobility and physiologic function (i.e. eating, defecation, urination and respiration) in the animal.

Multiple major survival surgeries should be avoided. However, there are instances when investigators will have a scientific need for the performance of multiple major survival surgical procedures. Such procedures must be described in the protocol, scientifically justified, and approved by the ACUC.

Recommendations for the performance of rodent surgery are based on the 1996 edition of the NIH Guide for the Use of Laboratory Animals and 9 CFR, **Animal Care Regulations**. Part 2, section 2.31(d)(1)(ix) of 9 CFR states that major survival surgical procedures on rodents "must be performed using aseptic procedures." This includes the use of sterile instruments, sterile surgical gloves, and aseptic preparation of the surgical site in order to prevent postoperative infections. A separate facility for rodent surgery is not necessary. A "sterile field" can be created by preparing a rodent surgical area in a room or portion of a room that is easily sanitized and not used for any other purpose during the time of the surgery.

1) Minimal procedures to be followed for non-survival surgeries

a) All animals should be sufficiently anesthetized prior to surgery such that they are completely unconscious and show no reaction to pain eliciting procedures. For most rodents, a lack of response to toe pinching is indicative of surgical anesthesia. Refer to the guidelines on the Animal Resources Program website for anesthesia for appropriate anesthetic agents and dosages. All animals are euthanized before recovery from anesthesia.

b) All personnel handling the animals must wear gloves.

c) Instruments and work surfaces need not be sterile but must be clean.

d) The surgical site should be free of hair.

e) Anesthetic and analgesic agents must be current (not expired). An exception is granted to use expired non-anesthetic or analgesic drugs for non-survival procedures and should be specifically labeled for this use.

2) Aseptic technique for survival surgeries

a) Surgery should be conducted on a clean, uncluttered lab bench or table surface. The surface should be covered with a clean drape.

b) Hair should be removed from the surgical site using clippers, hair plucking, or a depilatory agent. A complete sterile preparation includes wipes with an antiseptic scrub then removal with 70% isopropyl alcohol only glass bead or multiple sterile packs. This is performed three times followed by an application of antiseptic solution. Povidone iodine scrub and solution or chlorhexidine scrub and solution are most commonly used.

c) All instruments may be sterilized by the method of choice and on the basis of physical characteristics of the material to be sterilized. Acceptable techniques include autoclaving or cold sterilization. For autoclaving sterilization indicators (heat sensitive tape) should always be used to confirm that the materials reached the appropriate temperature. Additional scheduled biological monitoring is required to meet accreditation standards. Acceptable techniques for cold sterilization include soaking in 2% glutaraldehyde for 10 hours or 8% formaldehyde + 70% ethanol for 18 hours. There are several commercially available germicidal agents that are safer to use but attention must be paid to the shelf life of solutions once they are prepared. Gauze pads, intravascular catheters, suture material, etc. should also be sterile. Please note alcohol alone is not acceptable.

d) The surgeon should wash his/her hands with an antiseptic surgical scrub preparation and then aseptically put on gloves. If working alone, the surgeon should have the animal anesthetized and positioned and have the first layer of the double-wrapped instruments or cold pack opened before replacing putting on sterile gloves, so that he/she can remain sterile.

e) The surgeon should wear a facemask. A cap and gown or fresh labcoat are recommended but not required.

f) Ophthalmic ointment should be administered in both eyes to protect the corneas of the animal from drying and abrasion.

3) Multiple animal surgeries

a) If performing surgeries on more than one animal consecutively, start with a sterile instrument pack. The instruments can be placed in 70% ethyl alcohol in-between animals or in a glass bead sterilizer. The alcohol should be replaced when moderately contaminated with blood or other body fluids. Sterile gloves should be changed between surgeries. If the surgeon touches nonsterile surfaces, the surgeon may wipe his/her gloves for 30 seconds with sterile gauze pads soaked in alcohol.

4) Wound closure

a) The body wall should be closed with absorbable suture material. The skin should be closed with staples, wound clips, or with a nonabsorbable monofilament suture material in a simple interrupted pattern. Do not use silk or uncoated braided suture for skin closure, which acts as a wick to draw in

contamination. Skin sutures or staples must be removed by 7 to 10 days after surgery. The wound should be observed for swelling, heat, discharge and opening of the incision at least once daily for the first week, and appropriate action taken if a problem is noted.

5) Postoperative management

a) Rodents should be kept warm with an external heat source both during surgery and afterwards until ambulatory. A heating pad underneath the drape or a heat lamp can be used. Be careful when using a lamp not to overheat. Monitoring rectal body temperature is the best method to ensure that the animal is neither hypo- nor hyperthermic. Rodents under anesthesia are unable to adequately control their body temperature. Hypothermia significantly prolongs anesthesia and impairs recovery.

b) An analgesic agent should be administered prior to recovery from anesthesia. Preemptive pain management is much more effective than giving medication after observing signs of pain. Consultation with a veterinarian is advisable if you are unsure of the best choice for analgesia. Oral dosing in the drinking water is generally not an effective method, but may be useful in certain circumstances.

c) Antibiotics are not necessary if sterile technique is used. However, some procedures warrant the use of peri-operative antibiotics. This can be discussed with a veterinarian.

d) Once the animal is moving around, it may be returned to the vivarium.

e) At least once daily the animals should be observed for signs of pain, distress, incision problems. The following list (from *Recognition and Alleviation of Pain and Distress in Laboratory Animals*, NRC 1992) provides various signs to be watched for that would indicate acute pain in rodents.

- Decreased appetite. May eat bedding or their offspring.
- Decreased urine and fecal output.
- Decreased activity.
- Piloerection, ungroomed appearance.
- Excessive licking and scratching which may progress to self-mutilation.
- Abnormal stance or hunched posture.
- Respiration can be rapid and shallow with grunting or chattering on expiration.
- Pupils might be dilated.
- Porphyrin secretion ("red tears") might be seen around the eyes and nose.
- Vocalization.
- Increased aggressiveness when handled.

f) Group rRecords should be kept of all surgeries performed and any complications encountered. The surgical record should consist of: animal identification, location in which the surgery is performed, ACUC protocol number, the surgical procedure being performed, the person performing the surgery, the method of anesthesia and analgesia employed, the date/time and duration of the surgery and the time and duration of the anesthesia, and observations during the recovery period. If any of the above signs of pain or distress is observed, analgesia must be provide as described in the associated ACUC protocol. The veterinary staff in ARP is available for assistance. If the pain or distress is significant and untreatable, the animal will be euthanized.

#30 Guidelines for the Genotyping of Rodents

Purpose

The proper identification of genetically engineered animals is critical to research. Proper use of this procedure also allows a reduction in the number of animals required for experimentation. Normally, this procedure is performed on young rodents prior to weaning. With the advent of the Polymerase Chain Reaction (PCR) very little DNA is required for genotyping. Several tissues may be used as DNA sources including ear punches, fecal samples, oral or rectal swabs. Toe snipping is not sanctioned by the ACUC. If genotyping can be performed by PCR analysis investigators are encouraged to consider non-invasive DNA sources.

However some transgenic rodent experiments requires Southern analysis to identify copy numbers of transgenes or to discern rodents that homozygous from heterozygous for certain mutations. For these experiments, larger amounts of DNA are required. This increased DNA can be obtained from a tail biopsy. This procedure is safe, and causes minimum pain and distress when performed properly.

Tail Biopsy Regulations

1. Tail biopsy procedures should be described in all approved animal protocols.
2. Rodents should be between 10-21 days old for tail biopsy. At this young age, the tail tissue is soft and DNA yield will be most optimal. Also analysis at this age allows desired animals to be identified prior to weaning. If completed before 21 days anesthetic is not required as pain is transient and minimal. Rodents should be restrained in the hand and using either a sterile scalpel, razor blade, or scissors excise 5mm of the distal portion of the tail. From commercial kits it is possible to isolate ~50µg of DNA from this portion of a young rodent's tail. Between animals, the scalpel or scissors must be disinfected (it will also prevent cross contamination of samples). After the tissue slice has been removed, rodents must be monitored for excessive bleeding. If rodents do excessively bleed, then digital pressure should be applied to stop the bleeding. Animals should then be checked twice a week to ensure that tissue removal site has not become infected. If it has appropriate antibiotics should be applied.
3. For animals that are older than 21 days of age, a local or general anesthetic is required. Local anesthetic such as immersion of the tail in ice cold ethanol for 10 seconds or application of ethyl chloride spray. General anesthetics include halothane inhalation are permitted.
4. It is strongly discouraged for investigators to perform repeat excisions of tail tissue. Repeated biopsies must be justified when the protocol is submitted to the ACUC.

Approved 3/15/05

#31 Policy for bleeding of rodents

Purpose

Often it becomes necessary to examine the contents of the blood of experimental animals. In some cases, it is important to examine the same animal over an extended period of time to obtain data for longitudinal studies. The investigator is responsible for choosing the method of blood collection that provides the minimum amount of pain and distress for rodents. The most critical component of the bleeding process is the training and experience of the phlebotomist. Investigators are responsible for ensuring their personnel are appropriately trained in the method chosen. Training resources, include more experienced laboratory and veterinary personnel, and a variety of written and web-based resources including but not limited to the following:

<http://www.ahc.umn.edu/rar/blood.html>

<http://www.jhu.edu/animalcare/rat.htm#blood>

All blood sampling procedures should be included in approved protocols by the ACUC.

Blood volume and recovery guidelines:

When bleeding a rodent there are several major variables that must be considered: species, size, health status of rodent, quantity of blood needed and frequency of sampling. The quantity and frequency of blood sampling allowed is determined by the circulating blood volume of the animal. Table 1 (derived from the National Institutes of Health (NIH) guidelines) provides estimates of circulating blood volumes for rodents:

Table 1.
Circulating Blood
Volumes of
Rodents

Body Weight (g)	Circulating Blood Volume (ml)	1% (ml)	10% (ml)
20	1.1-1.140	.011-.014	.11-.14
25	1.37-.175	.014-.018	.14-.18
30	1.65-2.10	.017-.021	.17-.21
35	1.93-2.45	.019-.025	.19-.25
40	2.20-2.80	.022-.028	.22-.28
125	6.88-8.75	.069-.088	.69-.88
150	8.25-10.50	.082-.105	.82-1.0
200	11.00-14.00	.11-.14	1.1-1.4
250	13.75-17.50	.14-.18	1.4-1.8
300	16.50-21.00	.17-.21	1.7-2.1
350	19.25-24.50	.19-.25	1.9-2.5

Of the circulating volume, 10% can be removed every two weeks and 1% removed every 24 hours. Volumes greater than this should be justified in the protocol prior to approval by the ACUC and require fluid replacement. Table 2, derived from the NIH's blood collection guidelines, provides guidance on appropriate intervals (recovery periods) between blood collection in experiments involving multiple samples.

Table 2. Required Recovery Periods in Rodents Undergoing Single and Multiple Sampling

Single Sampling		Multiple Sampling	
% Circulatory Blood Removed	Approximate recovery period	% Circulatory Blood Removed in 24 hours	Approximate recovery period
7.50%	1 week	7.50%	1 week
10%	2 weeks	10-15%	2 weeks
15%	4 weeks	20%	4 weeks

Tail bleeding guidelines. This procedure can be used in both rats and mice. For small volumes (one-two drops) a distal portion of the tail (1-2mm) can be snipped by either razor blade, scalpel, or scissors. This method has the advantage that the clot/scab can be peeled for repeat bleed. Alternatively, the lateral tail vein or dorsal artery can be nicked by a perpendicular incision with a scalpel or razor blade. This method does not require anesthesia although restraint of the rodent is required. The ACUC recommends sampling of the vein, as arterial sampling is more rapid and is more difficult to maintain hemostasis. After bleeding animals, pressure should be applied to ensure that blood flow has stopped. Animals should be checked again within the hour to ensure that hemostasis is maintained. Additionally the animals should be checked daily to ensure there is no infection at the sampling site. If infection ensues, a veterinarian should be consulted.

Retro-orbital sinus/plexus bleeding guidelines. This method can be used in both mice and rats. However, it usually is not the method of choice for rats. When performed properly, the retro-orbital plexus/sinus is penetrated by a glass capillary tube or Pasteur pipette and blood is collected. This method should only be performed under general anesthesia, using injectable or inhalation anesthetics. It is recommended to alternate sampling between eyes and allow 10-14 days to pass between bleeding from the same orbit. Also, only sterile capillary tubes and pipettes should be used in this procedure to minimize the chances of periorbital infection. After bleeding mice should be checked to verify that clotting has occurred and that the eyes is not scarred. A veterinarian should be consulted if scarring or other abnormalities of the eye are observed.

Approved 3/15/05

WAKE FOREST UNIVERSITY
Photography/video permission form

Wake Forest University maintains an open policy regarding reasonable requests to visit its animal facilities, research and teaching laboratories; however, in order to protect the confidentiality of faculty research, to provide a minimally disruptive atmosphere for the residing animals and to guard against the misinterpretation of appropriate and humane policies and procedures, photography is not allowed except for official purposes that are approved as such by the Animal Care and Use Committee (ACUC). **Permission from the ACUC is required for government inspectors or news media to photograph/video animals at WFU. In certain cases, authorization may also involve approval by the Marketing and Public Relations Office and/or the Dean of the Medical School. The general public may not take photographs/videos at any time.**

Official permission is not required for faculty or their collaborators. WFU faculty may take pictures relating to the documentation of their own research/teaching programs. In addition, scientists/officials visiting the institution for professional reasons involving collaboration or information exchange may be allowed to take photographs that relate to the nature of their visit. The approval for such photography will rest with the professional judgment of the WFU faculty member who serves as host.

I, _____ (print name), am requesting permission to photograph/video animals within Wake Forest University (WFU) in accordance with the guidelines set forth in the WFU animal care and use policy, Photography/videotaping within Animal Facilities, Research and Teaching laboratories.

By my signature below, I acknowledge that:

- As a member of the news media, I agree to be accompanied by an official representative of the institution at all times and photography/video may be undertaken only with the expressed permission of said representative.
- As a government or private inspector, I agree to the following conditions:

1. Media may be taken only as an official part of the inspection and used only for documentation of items that cannot be documented in writing.
2. Prints of media used in a report must be provided to WFU for review and approval, as are all other components of site inspection reports.
3. All negatives and prints of media not used in an official report will be destroyed.
4. Media will not be distributed or used in any way other than as supporting evidence for an official site inspection report.
5. When media are used to document deficiencies, equivalent media taken by WFU may be used to document corrections and both will be used together in any report of ARP compliance with the Animal Welfare Act (AWA) provision.
6. Failure to comply with the above provisions will be considered by WFU as a breach of due process in USDA enforcement of the AWA and subject to legal redress.

Signature of photographer/videographer

Date

Address

Phone Number

FOR WFU USE ONLY – DO NOT WRITE BELOW THIS LINE

Signature of Animal Care and Use Committee, Chair

Signature of ARP Veterinarian

Date

Date

Appendix II ANIMAL RESOURCES PROGRAM SURGICAL CHART

Investigator _____ Phone# _____ Emergency# _____
 ACUC Protocol # _____ Animal Identification # _____
 Housing(Bldg/Rm) _____ Species _____ Body weight _____ kg
 Presurgical pain score* _____
 Surgical procedure (no abbreviations) _____

Premedication:

Drug(s) _____ Time _____ Dose(mg/kg) _____ Route _____

Induction:

Anesthetic _____ Time _____ Dose(mg/kg) _____ Route _____

Maintenance:

Anesthetic	Time	Amount	Comments

Additional analgesics and/or sedatives given prior to extubation:

Drug _____ Time _____ Dose(mg/kg) _____ Route _____

Name/Phone Numbers of Person(s) Responsible for postoperative recovery:

Name _____ Daytime _____ Weekend _____ Pager _____

Name _____ Daytime _____ Weekend _____ Pager _____

Postoperative drugs to be administered:

Drug _____ Dose(mg/kg) _____ Route _____ Frequency _____

Drug _____ Dose(mg/kg) _____ Route _____ Frequency _____

Recovery:

Time	Stage ⁺	Pain*	Observation

Surgical or anesthesia complications: no _____ yes _____ if yes, please explain:

* To assign a pain score refer to the pain score evaluation form. Postoperatively, assessments of pain should begin when the animals reach stage 3 of recover
 +Stage 4: Animal unconscious or semi-conscious and unable to sit or maintain sternal recumbancy. Stage 3: Animal conscious and can maintain sternal recumbancy to sit, but can not stand. Stage 2: Animal can stand and move about, is not eating and drinking normally. Stage 1: Animal is active, alert, eating and drinking normally; skin sutures are in place. Stage 0: Animal normal and skin sutures removed.

PAIN SCORE EVALUATION FORM**Pain Score**

0

movement.

Normal appearance and behavior

Heart rate and respiratory rate are normal

Analgesics may not be indicated.

1

Mild agitation, depressed and uninterested in surroundings, frequent position changes or reluctance to move

Mild changes in appearance, eyes partially closed

Decreased interaction

Heart and respiratory rate up to 30% above normal

Provide analgesics as described in the ACUC protocol.

For this level of pain, drugs in the anti-inflammatory class may be sufficient to relieve discomfort. (carprofen, ketoprofen)

2

Moderate agitation, restless and uncomfortable

Moderate changes in appearance, eyes sunken or glazed, unthrifty

Moderate changes in behavior, less mobile and less alert, unaware of surroundings, reluctance to move but will if coaxed

Heart rate and respiratory rate 30-45 % above normal

Extremely agitated, thrashing

Severe changes in appearance, eyes pale, enlarged pupils, guarding, hunched up appearance, legs in abnormal position, teeth grinding

Provide analgesics as described in the ACUC protocol.

For this level of pain, drugs in the Opioid agonist-antagonist class may be sufficient to relieve discomfort. (buprenorphine, butorphanol)

For additional analgesia, nonsteroidal anti-inflammatories (carprofen, ketoprofen) can be administered with an opioid agonist-antagonist

3

Heart and respiratory rate greater than 45 % above normal

Provide analgesics as described in the ACUC protocol.

For this level of pain, drugs in the Opioid agonist class may be sufficient to relieve discomfort. (morphine, hydromorphone)

For additional analgesia, nonsteroidal anti-inflammatories (carprofen, ketoprofen) can be administered with an opioid agonist.

Increase dose or frequency if pain continues

Observation

Asleep or awake, normal amount of

If signs of anxiety administer:
Sedative (diazepam, acepromazine)**Recommendations**

*Disclaimer: These are general guidelines describing criteria for assessing pain and recommendations for analgesia to alleviate the observed level of pain. It should be recognized that criteria indicative of pain will vary according to species and that there may be experimental contraindications associated with the recommended analgesics. Please consult the ARP veterinarians for specific drug dosages and combinations.

Appendix IV. Example Narrative of Search for Alternatives

Years searched:	1966-1999
Data Base (ex. Index Medicus)	Index Medicus
Keywords:	Thermoregulation, ear, microvasculature, Nerves, human, rabbit

Using the Index Medicus search engine, the keywords noted above yielded 267,000 entries. Searches using combinations of keywords ultimately yielded 57 entries. Each title and abstract (when available) was examined to see if the manuscript represented comparable work which would obviate the need for the proposed studies or offer an alternative to the use of animals in these experiments. Of the 57 entries, only 8 studies were directly related to the proposed work and none replaced or obviated the need to perform the experiments described.

WAKE FOREST UNIVERSITY
Animal Adoption Release Form

I, _____ (print name), am adopting the following animal from Wake Forest University (WFU):

Species: _____
Breed/strain: _____
Sex: _____
Color/markings: _____
Identification number: _____

By my signature below, I acknowledge that:

- *I am adopting this animal solely as a personal pet and not for the purpose of transferring the animal to a third party or for use in any illegal or harmful activity that may have adverse effects upon the animal or WFU.*
- *I understand that an examination of the animal has been conducted by a WFU Animal Resources Program (ARP) veterinarian. I have had an opportunity to ask the examining veterinarian health- and care-related questions and all questions have been answered to my satisfaction. I have inspected the animal myself and find it to be in good physical condition and suitable as a pet. I understand that there is no guarantee that the animal will remain healthy or is housebroken (as applicable).*
- *I am fully aware that this animal has been the subject of a research study at WFU, the specifics of which have been discussed between the examining veterinarian and me.*
- *I agree to accept full responsibility for this animal, including any and all future costs related to the animal's care and well being. I agree to comply with all applicable laws and ordinances for the municipality in which the animal is kept, and understand that WFU strongly recommends spaying or neutering the animal if it is a dog or cat.*
- *I assume the risks related to adoption of this animal and release Wake Forest University and its employees from all claims for damage to property or persons caused by this animal.*
- *I have completely and thoroughly read this Acknowledgement and agree that all terms are fully understood and voluntarily accepted for the purpose of making a full and final adoption of the above animal.*

Signature of Adopting Owner

Date

Address

Phone Number

FOR WFU USE ONLY – DO NOT WRITE BELOW THIS LINE

Signature of Principal Investigator
(Indicating Approval of Adoption)

Signature of ARP Veterinarian
(Indicating Examination of Animal)

Date

Date of Examination

Date of Release

Appendix VIII: Examples of animal number justification for studies involving cell culture of harvested tissue

Animal Care and Use Committee of Wake Forest University

Example 1.

A biochemical study of a metabolic pathway in cultured hepatocytes is proposed. Cultured hepatocytes are prepared from the liver of a rat; the cultures are viable for one week. There are 10 groups of experiments that need to be performed on the cultured hepatocytes using various inhibitors of the pathway under study. Previous experiments in that laboratory revealed a standard deviation of 19% in the activity of the metabolic pathway. The investigator needs to detect an effect of 25% on this pathway. Power calculation shows that 20 experiments per group (10 controls and 10 drug treatments per group) are required to detect this difference at the 5% level with a power of 80%. Thus, 200 successful experiments (10 groups x 20 experiments) are required for this study. Because experiments are sometimes unsuccessful due to technical difficulties, this number needs to be increased to 220. At an assumed performance rate of 10 metabolic experiments per week, the experiments would be completed in 22 weeks and thus require 22 rats for the weekly preparation of cultured hepatocytes. To account for the occasional unsuccessful culture of hepatocytes (about 10%), 24 rats (22+2) are requested for the proposed study.

Example 2.

The proposed study intends to examine growth rate and apoptosis in cultured myocytes. For the proposed experiments, a total of 10 timed, pregnant female rats are requested. Each female rat should deliver a litter of approximately 10 pups, based upon our prior experience using timed, pregnant female rats from Zivic-Miller. We will prepare both cultured myocytes and cardiac fibroblasts from the neonatal rat hearts, pooling the hearts from all of the pups in a single litter. Based upon previous studies in the literature (*full reference*), one 60 mm tissue culture dish or 1 cluster plate (either a 6, 12, or 24 well plate) of myocytes can be obtained per neonatal rat heart. Thus, from each litter of pups, 10 dishes of myocytes for individual experiments can be obtained.

In this pilot project, we request 2 litters of pups (2 adults and 20 pups) for initial studies, to learn the necessary techniques and obtain sufficient cells to characterize our preparations as to the percentage of myocytes per total number of cells (as an estimate of purity of the cultures). For the proposed experiments, a total of 8 litters of pups (8 adults and 80 pups) are requested. From the 10 available plates from each litter, 6 cluster plates will be used for growth assays (³H-thymidine incorporation, ³H-leucine incorporation, and cell number counts) and 4 plates will be used for the measurements of apoptosis (Annexin V and TUNEL assays).

Example 3.

To characterize the response to 10 different angiotensin peptides, dose-response curves will be generated including a control and increasing doses of each peptide from 10^{-11} to 10^{-4} M for a total of 9 different points, in duplicate, for each peptide (10 peptides x 9 concentrations x 2 duplicate samples or a total of 180 different determinations). To characterize the response to 5 different angiotensin peptide receptor antagonists with at least 5 of the 10 different peptides (assuming that 5 of the 10 proposed peptides cause an increase or a decrease in growth), experiments would include a submaximal concentration of the peptide, a 10-fold molar excess of the antagonist in the presence of the peptide, and a control with antagonist alone, in duplicate (3 experimental determinations x 5 angiotensin peptides x 5 peptide antagonists x 2 duplicate determinations or a total of 150 different determinations). For these studies we propose to use an n of 5 for each experimental determination. We have no prior experience with these exact types of studies in bone marrow cells making a power calculation difficult. However, based upon our previous experience measuring growth responses to angiotensin peptides in various

types of cultured cells including vascular smooth muscle cells, astrocytes, and various cell lines, we anticipate that this will provide us with statistically significant results. Thus a total of $180 + 150 \times 5$ or 1650 individual determinations will be required to complete the proposed experiments.

From a single mouse, using both femurs, approximately 3×10^7 total bone marrow cells can be isolated (based upon reported results in the literature). Based upon documentation from the manufacturer of the magnetic separation system that we propose to use, we anticipate a yield of approximately 1% Sca-1 cells from the total amount of bone marrow cells for a yield of 3×10^5 Sca-1 cells. For each individual experiment, we anticipate that we will need 1.5×10^4 Sca-1 cells per determination (per 1.2 ml of Methocult semisolid media selective for BFU-E growth (as indicated in the literature accompanying the Methocult growth conditions). Thus, 20 determinations can be conducted with the bone marrow isolated from a single mouse. To provide sufficient cells for the proposed studies, we would need 82.5 mice. Based upon a potential cell contamination rate of 10% (during the isolation of bone marrow cells), we request a total of 90 mice for the proposed studies, over a two-year time period.

Issued 7/02

Appendix IX: Table of Requirements by Species for the Occupational Health Program for Personnel Caring for or Using Laboratory Animals

Group	Species Used/Other Factors	PP Evaluation* & Annual Allergy Screening	PPD	Chest X-ray	Rabies Vaccine, Booster, & Titer	Diphtheria/Tetanus	Q-Fever Titer	Other
A	Small Animals - Rabbits, Rodents	Y				Every 10 years		
B	Dogs, Cats, Ferrets, Bats	Y			PP offer vaccine, titer recipients every 2 years. Booster if titer not protective.	Every 10 years		Toxoplasmosis: At time of PP or species addition, titer women of childbearing ability who work with <u>cats</u> . If negative, educate. Remove from lab if pregnant or planning to become pregnant.
C	Nonhuman Primates ⁺	Y Includes Biannual questionnaire with PPD positive individuals.	PP & every 6 months	If PPD positive**, PP & as recommended by physician		Every 10 years		Baseline serum collection: At PP or species addition.
D	Ruminants - Sheep [^] , Goats, Cattle	Y				Every 10 years	PP	
E	Other Farm Animals - Pigs	Y				Every 10 years		
F	Amphibians, Reptiles, Fish and Other Cold-blooded Animals	Y				Every 10 years		
G	Birds	Y				Every 10 years		
I	Infectious Disease Studies Class III or higher	Y				Every 10 years		Special consideration by Biosafety Committee

Y = Yes

PP = Pre-Placement

PPD = Tuberculin Skin Test, with Purified Protein Derivative

* Pre-Placement health evaluation includes pre-placement questionnaire, and allergy screening questionnaire.

** Personnel with PPD conversion, whether due to BCG vaccination or prior infection, must be confirmed non-infectious before entering a room housing nonhuman primates.

+ Nonhuman Primate Exposure includes those with exposure (in same room/area) to aerosolized particles from nonhuman primates.

^ Ruminant Exposure includes those with exposure (in same room/area) to aerosolized particles from sheep.

OVERCROWDED MOUSE CAGE EXEMPTION/EXTENSION REQUEST FORM

Instructions: For what condition are you requesting an exemption/extension?

- 1. To house more than 5 mice of weaning age or older per cage, **OR**
- 2. To house more than 1 litter per cage, **OR**
- 3. To house more than 2 adults and 1 litter per cage

Principal Investigator: _____ **Lab Contact:** _____

Protocol #: _____ **Account #:** _____

Location of mice: ROOM _____ RACK _____ CAGE(s) _____

Strain of mice: _____ Briefly describe the phenotype: _____

Scientific Justification for request: _____

(please attach any documentation supporting the justification claim)

Method for preventing double litters, if applicable (e.g. removal of the male prior to the birth of the first litter):

Weaning age requested, if applicable: (days or date): _____

Endpoint for Exemption/Extension, (days or date): _____

Principal Investigator _____ Date _____

ARP Signature _____ Date _____ Recommend Do Not Recommend

Comments: _____

ACUC Authorization _____ Date _____ Approve Do Not Approve

Comments: _____
