

**HANDBOOK FOR THE RESPONSIBLE CARE AND USE  
OF ANIMALS IN RESEARCH AND TEACHING**

**3<sup>rd</sup> EDITION, 2009**

**Minnesota State University MOORHEAD  
MOORHEAD, MN  
56563**

This handbook originally published in 1988, updated in 1991 and again in 1997, has served as the primary working document for the MSUM IACUC. The 1988 edition was based on that of the University of Wisconsin, Madison, permission given by Dr. James A. Will, DVM, Ph.D. This 2009 edition is intended to be a working document of the ARENA/OLAW GUIDEBOOK, 2002, and the USDA Bulletin, 9CFR, Ch. 1 (1-1-95 Edition), Animal and Plant Health Inspection Service (APHIS).

#### List of Abbreviations

AAALAC	American Association for Accreditation of Laboratory Animal Care
AALAS	American Association for Laboratory Animal Science
ACLAM	American College of Laboratory Animal Medicine
APHIS	Animal and Plant Health Inspection Service (a USDA office)
AVMA	American Veterinary Medical Association
AWA	Animal Welfare Act
AWI	American Welfare Institute
CEO	Chief Executive Officer
CI	Course Instructor
IACUC	Institutional Animal Care and Use Committee
IO	Institutional Official
ILAR	Institute of Laboratory Animal Resources
OLAW	Office of Laboratory Animal Welfare
PHS	Public Health Service
PI	Principal Investigator
NIH	National Institutes of Health
REAC	Regulatory Enforcement and Animal Care (an APHIS unit)
USDA	United States Department of Agriculture
WHO	World Health Organization

## PART 1

### I. Animal Welfare, A Perspective

The reader is directed elsewhere for historical and current perspectives on animal welfare, ethical considerations in use of animals in teaching and research as well as on alternatives to animal experimentation.

### II. Laws and Regulations

#### A. USDA Animal Welfare Act

The Laboratory Animal Welfare Act, commonly known as the Animal Welfare Act (AWA), Public Law 89-544, amended in 1970, 1976, and as the Food Security Act of 1985 (PL 99-198) is implemented by the USDA. The basic elements of this Act are discussed in section I. A., Historical Perspectives. A copy of this Act may be obtained by writing the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402. Title 9 of the Code of Federal Regulations (CFR) Parts 1, 2, and 3 deal with animal welfare. Part 1 deals with the definition of terms. Part 2 deals with regulations which includes licensing, registration, research facilities, attending veterinarian and adequate veterinary care, identification of animals, stolen animals records, compliance with standards and holding period, and miscellaneous. It also points out that the Animal and Plant Health Inspection Service (APHIS), an agency of the USDA is responsible for enforcing the regulations specified above through its Regulatory Enforcement and Animal Care (REAC) organizational unit.

The term animal in the amended AWA "means any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warm-blooded animal which is being used, or is intended for use for research, teaching, testing, experimentation, or exhibition purposes, or as a pet. This term excludes: Birds, rats of the genus Rattus and mice of the genus Mus bred for use in research, and horses and other farm animals, such as, but not limited to livestock or poultry, used or intended for use as food or fiber, or livestock or poultry used or intended for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber. With respect to a dog, the term means all dogs including those used for hunting, security, or breeding purposes." Animal dealers and exhibitors are required to be licensed. Research facilities are required to be registered. MSUM is registered with the USDA. **It is important to remember, however, that Minnesota State University Moorhead Animal Care and Use Policy covers all animals.**

#### B. AWA amendments

The 1985 amendments to the AWA resulted in additional responsibilities being imposed upon the research and educational communities. Subpart C of 9 CFR part 2 consists of all the regulations in Part 2 that are applicable to research/education facilities where live animals are used for research, tests, experiments or teaching. The following outline highlights some of the regulations established in subpart C:

### III. IACUC (Institutional Animal Care and Use Committee)

#### A. An IACUC must be established according to specific guidelines

1. the members must be appointed by the CEO
2. it must be composed of at least 5 individuals; A chair, individuals who meet specific stipulations which include active research, an individual representing a non science area, a veterinarian, an individual not affiliated with the University. An individual can satisfy more than one of these stipulations.

- B. Functions of IACUC
  1. the IACUC must review the facility's program every six months
  2. every six months, the IACUC must inspect the facility
  3. the IACUC must report to the IO every six months on the results of the review and inspection
  4. the IACUC must receive and review concerns from the public and personnel
- C. the IACUC shall make recommendations to the IO covering any aspect of the program
- D. the IACUC must review and approve or disapprove any proposed or ongoing animal care and use protocol as follows (The GUIDE, 2002):
  1. Rationale and purpose of the proposed use of animals.
  2. Justification of the species and number of animals requested. Whenever possible, the number of animals requested should be justified statistically.
  3. Availability or appropriateness of the use of less-invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see the ARENA/IACUC Guidebook, 2002, p 97).
  4. Adequacy of training and experience of personnel in the procedures used.
  5. Unusual housing and husbandry requirements.
  6. Appropriate sedation, analgesia, and anesthesia. (Scales of pain or invasiveness might aid in the preparation and review of protocols; see Appendix A, "Anesthesia, Pain and Surgery.")
  7. Unnecessary duplication of experiments.
  8. Conduct of multiple major operative procedures.
  9. Criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated.
  10. Postprocedure care.
  11. Method of euthanasia or disposition of animal.
  12. Safety of working environment for personnel.
- E. The IACUC shall suspend any inappropriate animal care and use activities.

**IV. Prior to the IACUC review**

- A. each IACUC member will be given a list of proposed activities to be reviewed
- B. written descriptions of all proposed activities involving the care and use of animals will be available to all IACUC members
- C. it will be determined if full committee review is necessary or if a subcommittee review is acceptable for the proposed activity's review
- D. it will be determined which IACUC members may be involved in the review process so that those members with conflicting interests are excluded from the review process
- E. the IACUC may consult with experts during the review process but the consultants will not have a vote

**V. Review results**

- A. the IACUC will notify the principal investigator/instructor of the review decision
  1. this notification will be written
  2. it will include a statement of reason for the decision
- B. if a proposal is disapproved, the investigator may respond to the committee
  1. the committee may reconsider its decision
- C. animal care and use activities will be reviewed annually

**VI. Suspension**

- A. the IACUC may suspend an activity if it deviates from the approved protocol
  1. the IO as well as the IACUC will be involved in the suspension process
  2. APHIS/OLAW will be notified

**VII. Approval**

- A. the IACUC's approval is necessary for all animal care and use
  - 1. additional university approval may also be required
- B. activity protocol must contain:
  - 1. the type of species and the approximate numbers of that species to be used
  - 2. the reason for using the animals and the appropriateness for using that species and number
  - 3. a complete description of the proposed use of the animals, including the methods that will be used to minimize pain, and the euthanasia method

**VIII. Personnel qualifications**

- A. it is the university's responsibility to ensure the personnel (scientists, research technicians, animal technicians, and other personnel) caring for and using animals are qualified through training and instruction
  - 1. the training and instruction shall include guidance in the following areas:
    - a. humane methods in animal maintenance and experimentation (basic needs, handling, general and surgical care)
    - b. reducing animal use (decreasing the numbers used)
    - c. the proper use of anesthetics and tranquilizers
    - d. proper reporting procedures involving abuse
    - e. the utilization of information resources

**IX. Veterinary care**

- A. the university will have established a formal agreement with a veterinarian to provide adequate veterinary care
  - 1. adequate veterinary care will include:
    - a. the availability of appropriate facilities, personnel, equipment, and services
    - b. the appropriate steps to ensure the general health and well-being of the animals, including weekends and holidays
      - (1) daily observation of all animals will be required to ensure their health and well-being but need not be carried out by the veterinarian
    - c. lines of communication in case of ill health
    - d. guidance in correct handling, restraining, and medicating procedures
- B. the attending veterinarian or appropriate designee will be a voting member of the IACUC

**X. Recordkeeping**

- A. the IACUC will keep:
  - 1. minutes of the IACUC meetings, which will include:
    - a. attendance
    - b. activities
    - c. deliberations
  - 2. protocols of proposed activities
  - 3. reports of review decisions
  - 4. semi-annual reports and recommendations, which are also forwarded to the IO
  - 5. procurement and disposal records for dogs and cats as well as additional marking details and forms as specified
- B. all reports and records will be kept for at least three years beyond closure
  - 1. they are available to the APHIS representatives
  - 2. reports and records will be confidential in so far as is consistent with state and federal regulations (Freedom of Information Act)

**XI. Annual report**

- A. the university will submit an annual report to APHIS, REAC Sector Supervisor for the State
  - 1. the report will cover the previous federal fiscal year
  - 2. the report will be submitted on or before December 1

3. the report must be signed by the IO or the CEO
4. the report shall:
  - a. ensure that professionally acceptable standards in the care and use of animals were employed
  - b. ensure alternative procedures were considered
  - c. ensure that the university is adhering to the standards and regulations under the Act
  - d. include the location of all animals used in the approved activities
  - e. include the type and numbers of animals used for each category of activities
  - f. include the type and numbers of animals housed but not yet utilized in approved activities
5. a similar report will be submitted to OLAW on or before January 31 to cover the previous fiscal year, if the institution has an active NIH/OLAW Assurance.

## **XII. Miscellaneous**

- A. APHIS will have the right to access and inspect the university's animal care and use records and facilities during business hours
- B. APHIS has the right to document their inspections through photography and other means
- C. if abuse is found, APHIS has the right to require and effect the appropriate changes
- D. APHIS will publish a list of all registered research facilities
- E. local authorities shall be allowed to inspect the facilities for the purpose of finding missing animals

## **XIII. AWA Requirement**

- A. Under the AWA, each research/education facility using covered animals is required to submit an annual report covering the period from October 1 of the preceding year through September 30 of the current year. This report must list the species and numbers of animals used during that period and whether or not pain or distress was involved in their use. As a result, all animal users at MSUM are required to turn in their reports annually to IACUC with this information by November 1 .

1. The USDA conducts periodic inspections of registered research facilities to assure compliance with all requirements of the AWA. There are specific standards for care, shelter, cage size, facility structure, temperature, ventilation, lighting, drainage, feeding, watering, sanitation, veterinary care, and transport. Any suffering animal may be treated or destroyed by the veterinary inspector after a reasonable effort to notify the researcher. Any management or structural deficiencies found will be cited and a specified period of time be given for correction. In the event of such a citation, the IACUC veterinarian associated with the facility will notify the necessary departmental personnel and assist in responding to the USDA.

- B. Import, Export and Wildlife Laws

Laws governing the import or export of animals are administered by the Center for Disease Control, the USDA, the U.S. Fish and Wildlife service of the Department of the Interior, and the Office of Marine Mammals and Endangered Species of the National Oceanic and Atmospheric Administration. For further information, consult the GUIDE, 2002.

- C. Public Health Service Policy

The Public Health Service (PHS) of the U.S. Department of Health and Human Services requires research institutions receiving PHS funds to conform to the PHS Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions. NOTE: The Animal Welfare Assurance (No. A3491-01) of MSUM was classified as inactive on May 5, 1997 and was reinstated July 22, 2008 (NO. A-4566-01) by OLAW. The PHS Policy covers "Any

live, vertebrate animal used or intended for use in research, research training, experimentation or biological testing, or for related purposes." The PHS requires institutions to use the NIH Guide for the Care and Use of Laboratory Animals as the basis for developing and implementing an institutional program for activities involving animals. The basic requirements of the Policy include:

1. Institutional submittal of a detailed **Animal Welfare Assurance Statement** describing the institutional program for animal care and use, the lines of authority for the program, and the make-up and responsibilities of the Institutional Animal Care and Use Committee (IACUC).
2. Establishment of animal care and use committees which are required to review proposals involving animal use for compliance with the AWA, the NIH Guide, and the AVMA Panel on Euthanasia, for personnel qualifications, avoidance of pain and distress, and appropriate use of anesthetics and analgesics.
3. The committees are also required to "review the care and treatment of animals in all animal study areas and facilities of the research entity and to conduct semiannual inspections to evaluate compliance."
4. Minnesota State University Moorhead Animal Care and Use Committee (IACUC) structure is described under part II of this handbook.

#### **XIV. Public Health Services Policy**

- A. The Public Health Service Policy is intended to implement and supplement the United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training which was developed by the Interagency Research Animal Committee. Nine federal agencies have adopted the U.S. Government Principles, including the Department of Health and Human Services, of which PHS is a part. These principles are as follows:
  1. The development of knowledge necessary for the improvement of the health and well-being of humans, as well as other animals, requires in vivo experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible Institutional Official shall ensure that these principles are adhered to:
    1. The procurement, transportation, care, and use of animals should be in accordance with the Animal Welfare Act and other applicable federal laws, guidelines and policies.
    2. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge or the good of society.
    3. The animals selected for a procedure should be of an appropriate species and quality. The minimum number required to obtain valid results should be determined by statistical analysis. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.
    4. Proper use of animals, including the avoidance and minimizing of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

5. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

6. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if necessary, during the procedure.

7. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling and use of the species being studied. In any case, veterinary care shall be provided as needed.

8. Investigators and other personnel shall be appropriately trained and experienced for conducting procedures on living animals. Adequate arrangements shall be made for in-service training, including the proper and humane care and use of laboratory animals.

9. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an Institutional Animal Care and Use Committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

#### XV . **NIH Guide for the Care and Use of Laboratory Animals (the GUIDE, 2002).**

A. "The purpose of the Guide is to assist institutions in caring for and using laboratory animals in ways judged to be professionally and humanely appropriate. The recommendations are based on published data, scientific principles, expert opinion, and experience with methods and practices that have proven to be consistent with high quality, humane animal care and use."

B. The Guide for the Care and Use of Laboratory Animals has detailed sections on institutional policies, including animal care and use committee responsibilities and animal protocol review, laboratory animal husbandry, veterinary care, physical plant specifications, and special considerations such as hazardous agents and farm animals. Although marine mammals and cold-blooded animals are not specifically discussed, the humane principles stated are applicable to their care and use as well. The discussion on husbandry and management in this handbook is based on the Guide and material from the Canadian Council on Animal Care. The Guide was revised in 2002; a copy of the Guide may be obtained by writing to: Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402. Ask for GPO Publication #0-309-05377-3. The Health Research Extension Act of 1985, PL 99-158, which reauthorized funding for the NIH to put the **force of law** behind the Guide. The Guide is an essential document for anyone involved with laboratory animal care and use. (A copy of the Guide is on reserve at the library.)

C. Canadian Council on Animal Care Policy

The MSUM IACUC also supports the principles embodied in the Canadian Council on Animal Care as to "Ethics of Animal Experimentation" (1989), summarized below:

1. In studies involving animals there must be reasonable expectation that such studies will contribute significantly to knowledge which may eventually lead to the improvement of the health and welfare of either humans or animals.

2. Investigators have a moral obligation to abide by the humanitarian dictate that experimental animals are not to be subjected to unnecessary pain or distress.
3. If pain and distress are necessary concomitants of the experimental procedure, then these should be minimized both in intensity and duration.
4. An animal that is observed to be in a state of severe pain which cannot be alleviated should be immediately destroyed, using a humane, acceptable method for euthanasia which must include, as an initial action, rapid induction of unconsciousness.
5. Biological testing may require continuation until the death of the experimental subject. This requirement, in the face of distinct and irreversible signs that severe pain or distress is being observed, would clearly violate the principles outlined above. In such cases, alternative end points should be sought which would not only satisfy the objectives of the study but also give humane consideration to the animal.
6. In test procedures, the investigator should be especially cautious with tests which may cause pain and distress. Acceptance should not be based on low cost and ease of application.
7. Experiments involving the withholding of food and water should be short-term, and have no lasting detrimental effect on the health of the animal.
8. Prolonged physical restraint procedures which result in distress or ill effects should only be used after alternative procedures have been considered and found inadequate.
9. The use of painful experiments solely for the demonstration of established scientific knowledge cannot be justified.
10. It is accepted that where the animal is anesthetized and insensitive to pain during an entire experimental procedure and euthanized before regaining consciousness, there is no conflict of opinion concerning the acceptability of experimental procedures as long as the studies abide by the principles enunciated in this document. This applies also to the conduct of experiments involving no pain or distress to the animal. However, in the use of conscious animals, an informed assessment of the degree of pain and its duration is required, in order to relate these to acceptable limits. Investigators must be especially prudent in their use of the following procedures:
  - a. Experiments involving withholding pre- or post- operative analgesic medication.
  - b. Paralyzing and immobilizing experiments where there is no reduction in the sensation of pain.
  - c. Extreme environmental conditions such as low or high temperatures, high humidity, or modified atmospheres.
11. It must be understood that the degree of pain involved should never exceed that determined by the humanitarian importance of the problem to be solved by the experimental study. The following procedures must be restricted and used only when, on the basis of expert opinion, it is anticipated that their utilization will undoubtedly contribute knowledge or benefit to humans or animals:
  - a. Prey killing or fighting experiments.
  - b. Experimental burn or fracture studies.
12. Certain experimental procedures are known to inflict excessive pain and are thus unacceptable. These include:

- a. Utilization of muscle relaxants or paralytics such as curare or succinylcholine alone, without anesthetics, during surgical procedures.
- b. Traumatizing procedures involving crushing, striking or beating an unanesthetized animals or animals allowed to recover from anesthesia.

**XVI. American Veterinary Medical Association Panel on Euthanasia**

- A. Both the PHS Policy and the NIH Guide refer to the AVMA Panel on Euthanasia (2001) as the recommended guide for use when choosing an euthanasia method for warm-blooded animals. Exceptions to the stated recommendations in this report are acceptable only if scientifically justified and only after review by the attending veterinarian and the Institutional Animal Care and Use Committee. Copies of this report may be obtained via reprint request or by calling the IACUC chairperson. Acceptable euthanasia methods are more fully discussed in Appendix B.

**XVII. Minnesota State University Moorhead**

**A. Minnesota State University Moorhead Policy:**

**All animals used for teaching, research, or other activities on campus and at satellite facilities shall be used and cared for according to the principles stated in the ARENA/OLAW Institutional Animal Care and Use Committee Guidebook (2002) (The Guide), regardless of the species or source of funds used to conduct teaching, research, or other activities.**

**B. Animal Care and Use Committee**

MSUM has an Institutional Animal Care and Use Committee (IACUC), which meets the membership requirements of the AWA, the PHS Policy, the NIH Guide, (2002), and the OLAW Manual, (2002) minimum of five members. They include: the attending veterinarian, responsible for the facility's program of animal care and use; a member whose primary interest is non-scientific; a member who is a practicing scientist experienced in research involving animals; and a member not associated with the university. The committee is appointed by the chief executive officer, or his/her designee. The IACUC promulgates the animal care and use policy, and functions as an appeals committee for animal concerns. The responsibilities of the Institutional Animal Care and Use Committee, as dictated, by the PHS policy are listed below. The PHS/OLAW and USDA/APHIS policies, while overlapping significantly, do have several differences.

**Part 2**

**Responsibilities of Animal Care Committee**

**(Excerpted from ARENA/OLAW publication, "Public Health Service Policy on Humane Care and Use Laboratory Animals", 2002)**

- I. **Formation of an Institutional Animal Care and Use Committee (IACUC) and application for NIH/OLAW Assurance.**
  - A. **The Chief Executive Officer shall appoint an Institutional Animal Care and Use Committee (IACUC), qualified through the experience and expertise of its members, to oversee the institution's animal program, facilities, and procedures.**

**B. Application for a NIH Animal Welfare Assurance must include the names, position titles, and credentials of the IACUC chairperson and the members. The committee shall consist of not less than five members, and shall include at least:**

- (1) one doctor of veterinary medicine, with training or experience in laboratory animal science and medicine, who has direct or delegated program responsibility for activities involving animals at the institution;**
- (2) one practicing scientist experienced in research involving animals;**
- (3) one member whose primary concerns are in a non-scientific area (for example, ethicist, lawyer, member of the clergy); and**
- (4) one individual who is not affiliated with the institution in any way other than as a member of the IACUC, and is not a member of the immediate family of a person who is affiliated with the institution.**
- (5) An individual who meets the requirements of more than one of the categories of this Policy may fulfill more than one requirement. However, no committee may consist of less than five members.**

**C. Functions of the Institutional Animal Care and Use Committee (IACUC)**

**As an agent of the institution, the IACUC shall with respect to PHS-conducted or supported activities:**

- 1. review at least once every six months the institution's program for humane care and use of animals, using the Guide as a basis for evaluation;**
- 2. inspect at least once every six months all of the institution's animal facilities (including satellite facilities) using the Guide as a basis for evaluation;**
- 3. prepare reports of the IACUC evaluations conducted as required in 1. and 2. above, and submit the reports to the Institutional Official. The Institutional Animal Care and Use Committee (IACUC) may, at its discretion, determine the best means of conducting an evaluation of the institution's programs and facilities. The IACUC may invite ad hoc consultants to assist in conducting the evaluation. However, the IACUC remains responsible for the evaluation and report. (NOTE: the reports shall be updated at least every six months upon completion of the required semiannual evaluations and shall be maintained by the institution and made available to USDA/OLAW upon request. The reports must contain a description of the nature and extent of the institution's adherence to the Guide and this Policy and must identify specifically any departures from the provisions of the Guide and this Policy, and must state the reasons for each departure. The reports must distinguish significant deficiencies from minor deficiencies. A significant deficiency is one which, consistent with this Policy, and, in the judgment of the IACUC and the Institutional Official, is or may be a threat to the health or safety of the animals. If program or facility deficiencies are noted, the reports must contain a reasonable and specific plan and schedule for correcting each deficiency. If some or all of the institution's facilities are accredited by AAALAC or another accrediting body recognized by PHS, the report should identify those facilities as such).**
- 4. review concerns involving the care and use of animals at the institution;**

5. make recommendations to the Institutional Official regarding any aspect of the institution's animal program, facilities, or personnel training;
6. review and approve, require modifications in (to secure approval) or withhold approval of those components of PHS-conducted or supported activities (protocols) related to the care and use of animals as specified in C. of this Policy;
7. review and approve, require modifications in (to secure approval), or withhold approval of proposed significant changes regarding the use of animals in ongoing activities (protocols) and
8. be authorized to suspend an activity involving animals in full quorum meeting by a vote of the majority of the committee.

**D. Review of PHS-Conducted or Supported Research Projects**

1. In order to approve proposed research projects or proposed significant changes in ongoing research projects, the IACUC shall conduct a review of those components related to the care and use of animals and determine that the proposed research projects are in accordance with this Policy. In making this determination, the IACUC shall confirm that the research project will be conducted in accordance with the Animal Welfare Act insofar as it applies to the research project, and that the research project is consistent with the Guide unless acceptable justification for a departure is presented. Further, the IACUC shall determine that the research project conforms with the institution's Assurance and meets the following requirements:
  - a. Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design.
  - b. Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator.
  - c. Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly sacrificed at the end of the procedure or, if appropriate, during the procedure.
  - d. The living conditions of animals will be appropriate for their species and contribute to their health and comfort. The housing, feeding, and nonmedical care of the animals will be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied.
  - e. Medical care for animals will be available and provided as necessary by a qualified veterinarian.
  - f. Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained on those procedures.
  - g. Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (2001) or succeeding revised editions, unless a deviation is justified for scientific reasons in writing by the investigator.

2. Prior to the review, each IACUC member shall be provided with a list of proposed research projects to be reviewed. Written descriptions of research projects that involve the care and use of animals shall be available to all IACUC members, and any member of the IACUC may obtain, upon request, full committee review of those research projects. If full committee review is not requested, at least one member of the IACUC, designated by the chairperson and qualified to conduct the review, shall review those research projects and have the authority to approve, require modifications in (to secure approval) or request full committee review of those research projects. If full committee review is requested, approval of those research projects may be granted only after review at a convened meeting of a quorum of the IACUC and with the approval vote of a majority of the quorum present. No member may participate in the IACUC review or approval of a research project in which the member has a conflicting interest (e.g., is personally involved in the project) except to provide information requested by the IACUC; nor may a member who has a conflicting interest contribute to the constitution of a quorum.
3. The IACUC may invite consultants to assist in the review of complex issues. Consultants may not approve or withhold approval of an activity or vote with the IACUC unless they are also members of the IACUC.
4. The IACUC shall notify investigators and the institution in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications required to secure IACUC approval. If the IACUC decides to withhold approval of an activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in person or in writing.
5. The IACUC shall conduct continuing reviews of activities covered by this Policy at appropriate intervals as determined by the IACUC, but not less than once every three years.
6. The IACUC may suspend an activity that it previously approved if it determines that the activity is not being conducted in accordance with applicable provisions of the Animal Welfare Act, the Guide, the institution's Assurance, or the above criteria of this Policy. The IACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present.
7. If the IACUC suspends an activity involving animals, the Institutional Official, in consultation with the IACUC, shall review the reasons for the suspension, take appropriate corrective action, and report that action with a full explanation to OLAW.
8. Applications and proposals that have been approved by the IACUC may be subject to further appropriate review and approval by officials of the institution. However, those officials may not approve an activity involving the care and use of animals if it has not been approved by the IACUC.

**E. Information Required in Applications and Proposals for Awards Submitted to PHS**

**1. All Institutions**

Applications and proposals (competing and non-competing) for awards submitted to PHS that involve the care and use of animals shall contain the following information:

- a. identification of the species and approximate number of animals to be used;

- b. rationale for involving animals, and for the appropriateness of the species and numbers to be used;
- c. a complete description of the proposed use of the animals;
- d. a description of procedures designed to assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research, and that analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals; and a description of any euthanasia method to be used.
- e. Non-competing applications and contract proposals for other than full and open competitions need not repeat the information required by D.1.a-e. if the information was complete in the last competing application or proposal and there are no significant changes to that information. However, the application or proposal must contain a statement to that effect. If there are significant changes in the information, then the application or proposal must specifically identify them and state the reasons for the changes.

**2. Institutions That Have an Approved or Provisionally- Acceptable Assurance**

Applications or proposals (competing and non-competing) covered by this Policy from institutions which have an approved or provisionally-acceptable Assurance on file with OLAW shall include verification of approval (including the date of the most recent approval) by the IACUC of those components related to the care and use of animals. With the authorization of PHS, such verification may be filed at a time not to exceed 60 days after submission of applications or proposals. Until further notice, PHS hereby authorizes all institutions with approved or provisionally-acceptable Assurances to file verification of IACUC approval either along with the competing application or proposal or within 60 days of submission of the application or proposal. From time to time PHS will re-evaluate this blanket authorization. Any decision to withdraw this authorization will take place only after ample opportunity is provided for comment by the public.

If verification of IACUC approval is submitted subsequent to the submission of the application or proposal, the verification shall state the modifications, if any, required by the IACUC. The verification shall be signed by an individual authorized by the institution, but need not be signed by the Institutional Official.

**3. Institutions That Do Not Have an Approved or Provisionally-Acceptable Assurance**

Applications and proposals covered by this Policy from institutions that do not have an approved or provisionally-acceptable Assurance on file with OLAW shall contain a declaration that the institution will establish an IACUC and submit an Assurance upon request by OLAW. After OLAW has requested the Assurance, the institution shall prepare an Assurance in accordance with A. of this Policy and the established IACUC shall review those components of the application or proposal as required by C. of this Policy. The institution shall then submit to OLAW the Assurance and verification of IACUC approval. The verification shall state the modifications, if any, required by the IACUC. The verification shall be signed by an individual authorized by the Institutional Official who signed the Assurance.

**F. Record Keeping Requirements**

1. The awardee institution shall maintain:
  - a. an Assurance which has been either approved or deemed provisionally-acceptable by the PHS;
  - b. minutes of IACUC meetings, including records of attendance, activities of the committee, and committee deliberations;
  - c. records of applications, proposals, and proposed significant changes in the care and use of animals and whether IACUC approval was given or withheld;
  - d. records of semiannual IACUC reports and recommendations (including minority views) as forwarded to the Institutional Official; and
  - e. records of accrediting body determinations.
2. All records shall be maintained for at least three years; records that relate directly to applications, proposals, and proposed significant changes in ongoing activities reviewed and approved by the IACUC shall be maintained for the duration of the activity and for an additional three years after completion of the activity. All records shall be accessible for inspection and copying by authorized OLAW or other PHS representatives at reasonable times and in a reasonable manner.

**G. Reporting Requirements**

1. At least once every 12 months, the IACUC, through the Institutional Official, shall report in writing to USDA/OLAW;
  - a. any change in the institution's program or facilities which would place the institution in a different category than specified in its Assurance;
  - b. any change in the description of the institution's program for animal care and use.
  - c. any changes in the IACUC membership; and
  - d. notice of the dates that the IACUC conducted its semiannual evaluations of the institution's program and facilities and submitted the evaluations to the Institutional Official.
2. At least once every 12 months, the IACUC, at an institution which has no changes to report shall submit a letter, through the Institutional Official, to USDA/OLAW stating that there are no changes and informing USDA/OLAW of the dates of the required IACUC evaluations and submissions to the Institutional Official.
3. The IACUC, through the Institutional Official, shall promptly provide OPRR/OLAW with a full explanation of the circumstances and actions taken with respect to:
  - a. any serious or continuing noncompliance with this Policy;
  - b. any serious deviation from the provisions of the Guide; or
  - c. any suspension of an activity by the IACUC.

4. **Reports filed under F. of this Policy shall include any minority views filed by members of the IACUC.**

**(End of PHS excerpt)**

Since the Committee chairperson and membership change periodically; please contact the Institutional Official (IO), currently the Dean of Social and Natural Sciences

4. Animal Use Protocols

**All Minnesota State University Moorhead projects using animals covered by APHIS/OLAW Guidelines must have an animal use protocol reviewed and approved by the IACUC. All projects must be classified as to their invasiveness and the amount of pain they may cause.** The "Classification for Research Animal Use" included here with the protocol gives the various categories used by Minnesota State University Moorhead. Protocols are reviewed for humane care and use, appropriate species and number, adequate anesthesia and analgesia, qualifications of personnel, licensure (Federal, DEA and State of Minnesota, MBP) to use controlled substances and other areas as needed. The purpose of the review is not to evaluate scientific merit. Each protocol is assigned a unique protocol code number. This protocol code number must be displayed with the project animals in some manner. Animal identification should be adequate to identify single animals in the case of dogs, cats and large or unique animals. Investigators and educators who are found to be doing research or using vertebrate animals for education without an approved protocol form on file will receive written notification to submit a protocol form to IACUC. If the investigator does not comply by the specified deadline, the IACUC and then the University President will be notified and appropriate action taken. The Revised Animal Use Protocol Review Form (4/2004) is included here. **Copies of the Animal Use Protocol form may be obtained from the IACUC Chairperson or the IACUC webpage ([www.mnstate.edu/iacuc](http://www.mnstate.edu/iacuc)).**

Animal Use Protocol Review forms are to be completed in full and in detail, signed by the principal investigator/course instructor and the department chairperson, and then submitted to the IACUC Chairperson. Any IACUC member may be consulted for assistance in completing the form. Each protocol is assigned a protocol code number, and the IACUC questionnaire/verification of all personnel, including the principal investigator/course instructor and individuals performing surgery, is checked, a copy is filed, and the protocol is sent for review. When approved, the committee chairperson signs a "verification of approval" letter, which is sent to the principal investigator/course instructor and a copy to the department chair. Generally the principal investigator must send this letter to the grant funding agency as evidence of IACUC approval. **Annual personnel and protocol updates are necessary, and complete re-submittal is required every three years. However, if there are changes in the protocol, i.e., surgical to nonsurgical procedures, personnel, animal usage, etc., before the update notification is received, a protocol amendment must be submitted.** Examples of protocol application and protocol amendment forms follows. However, the protocol amendment form can only be used for minor protocol changes (eg. Adding, dropping species and/or researchers). Major protocol changes require resubmission of the protocol for approval by the IACUC.



- Breeding colonies of any animal species (USDA does not require listing of rats, mice, birds) that are held in legal sized caging and handled in accordance with the Guide and other applicable regulations. Breeding colony includes parents and offspring.
- Newly acquired animals that are held in proper caging and handled in accordance with applicable regulations.
- Animals held under proper captive conditions or wild animals that are being observed.

**Classification C:**\_\_\_\_\_ Animals upon which teaching, research, experiments, or tests will be conducted involving no pain, distress, or use of pain-relieving drugs.

**Examples:**

- Procedures performed correctly by trained personnel such as the administration of electrolytes/fluids, administration of oral medications, blood collection from a common peripheral vein per standard veterinary practice (dog cephalic, cat jugular) or catheterization of same, standard radiography, parenteral injections of non-irritating substances.
- Euthanasia performed in accordance with the recommendations of the most recent AVMA Panel on Euthanasia, utilizing procedures that produce rapid unconsciousness and subsequent humane death.
- Manual restraint that is no longer than would be required for a simple exam; short period of chair restraint for an adapted nonhuman primate.

**Classification D:**\_\_\_\_\_ Animals upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used.

**Examples:**

- Surgical procedures conducted by trained personnel in accordance with standard veterinary practice such as biopsies, gonadectomy, exposure of blood vessels, chronic catheter implantations, laparotomy or laparoscopy.
- Blood collection by more invasive routes such as intracardiac or periorbital collection from species without a true orbital sinus such as rats and guinea pigs.
- Administration of drugs, chemicals, toxins, or organisms that would be expected to produce pain or distress but which will be alleviated by analgesics.

**Classification E:**\_\_\_\_\_ Animals upon which testing, experiments, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.

**Examples:**

- Procedures producing pain or distress unrelieved by analgesics such as toxicity studies, microbial virulence testing, radiation sickness, and research on stress, shock, and pain.
- Surgical and postsurgical sequella from invasion of body cavities, orthopedic procedures, dentistry or other hard or soft tissue damage that produces unrelieved pain or distress.
- Negative conditioning via electronic shocks that would cause pain in humans.
- Chairing of nonhuman primates not conditioned to the procedure for the time period used.

**NOTE REGARDING CLASSIFICATION E:** An explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anesthetic, analgesic, or tranquilizing drugs must be provided on Attachment 1. This form must accompany USDA Form 7023 to support any Federal Grant application with a procedure Classification E listings.

**3. ANIMAL CHARACTERISTICS:** The investigator should state the required number of animals to be used in the research project.

Species	Sex	Age/Weight	Animal Vendor	Location of Housing	Total

**4. BIOHAZARDOUS MATERIAL:** If the animal use involves biohazardous materials the appropriate category should be checked and approval obtained from other required review committees:

Infectious agents \_\_\_ **Carcinogens** \_\_\_ **Radioisotopes** \_\_\_ **Recombinant DNA**

**Other** \_\_\_\_\_

**SECTION II**

**1. Purpose of the Study:**

**2. Potential Value of the Study:**

**3. Alternatives to Animal Use:**

**4. Species Justification:**

**5. Justification of the Number of Animals Requested Based Upon Experimental Design:**

**6. Current IACUC Approved Protocol(s)**  
**[Attachments]**

**7. Procedures:**

**8. Alternatives to Painful Procedure(s):**

**9. Restraints:**

Surgery to be performed: Yes \_\_\_ No \_\_\_

**A. Building in which surgery will be performed:**

**B. Personnel who will perform surgery and type/length of training/experience:**

**C. Briefly describe surgical procedures:**

10. Pain Control During the Procedure(s) (anesthetic method, drugs, routes of administration, supplementation, schedules):
11. Estimation of Potential Postoperative/Intervention Pain:  
 Multiple surgery on one animal: Yes \_\_\_\_ No \_\_\_\_  
 Justify:
12. Post-Procedure/Chronic Care:  
 A. Post-Procedure Monitoring:  
 B. Criteria for Pain:  
 C. Analgesic(s):  
 D. Antibiotics:
13. Describe all non-surgical manipulations or procedures involving the animal (e.g., toe clipping, transmitters, banding, drug administration, blood collection, diet change):
14. Specify duration of procedures (Insert or attach your description if more room is needed):
15. Will an adjuvant be used? Yes \_\_\_\_ No \_\_\_\_  
 Comments:
16. Give total volume of adjuvant to be used, number of injection sites per dosage and number of doses:
17. Will pristane or other abdominal irritants in mice be used? Yes \_\_\_\_ No \_\_\_\_  
 If yes, list the dosages:
18. Will blood be drawn? Yes \_\_\_\_ No \_\_\_\_  
 If yes, how frequently and what volume of blood or ascites will be taken:
19. Give building and room in which procedure described in 17 and 18 will be conducted:  
 Building \_\_\_\_\_ Room \_\_\_\_\_
20. Investigator(s) Qualifications/Experience (indicate principal or secondary investigators):  
 A. Knowledge of Species:  
 B. Relevant Experience:  
 C. Responsibilities:  
 D. Investigator(s) have taken, signed and/or completed the IACUC exam: Yes \_\_\_\_ No \_\_\_\_

21. Provisions made to ensure personnel and students entering the project at a later date will be properly trained and informed: Yes\_\_\_\_ No\_\_\_\_  
 A. Comments:
22. Person(s) or unit responsible for animal husbandry (daily care) and location of animal facilities:
23. Veterinarian responsible for veterinary care if other than the IACUC vet (Dr. Merrill Reinhiller, DVM):  
 Name:  
 Address:  
 Phone Number:
24. Describe the end point of the research at which the animal will be euthanized:
25. Describe the method(s) of euthanasia that will be used during or at the conclusion of the project including agents, dosages, and routes of administration:

### SECTION III

1. Will this study use animals caught in the wild? Yes\_\_\_\_ No\_\_\_\_  
 (If no, skip to section IV)
2. When, where and how will the animals be trapped?
3. What type of traps will be used?
4. How often will the traps be checked?
5. How will the safety (physical, diseases) of the investigator be ensured while retrieving live animals?
6. If the animal will be brought to campus/satellite facility, what precautions will be taken to prevent disease (if present) from transmitting to other animals or humans?
7. Where will the animals be housed on campus?  
 A. Building  
 B. Room
8. References:



# Attachment 1

## Explanation for USDA Classification E

(This report is required to accompany USDA Form 7023 to support any USDA Classification E listings.)  
This document must be typed.

Name of investigator:

Animal Study Proposal Title:

Species and number of animals listed in Classification E for each year:

Species:

Number of animals:

Year 1-

Year 2-

Year 3-

Total:

Description of project including reason(s) for species selection:

Provide a scientific justification to explain why the use of anesthetics, analgesics, sedatives or tranquilizers during and/or following painful or distressing procedures is contraindicated:

Signature of investigator:

Date:

Signature of IACUC Chairperson:

Date:

**MINNESOTA STATE UNIVERSITY MOORHEAD  
ANIMAL USE PROTOCOL REVIEW SUPPLEMENT REQUIRED  
BY THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

**In the event of an emergency at the animal research facility, it may be necessary for a University spokesperson to describe the nature of the research taking place in that facility. It is important to have this information available quickly, even if the Principal Investigator/Course Instructor cannot be reached. Therefore, we ask that all P.I.'s/Course Instructors provide a brief (approximately one page) description, understandable to the layperson, of the research going on in his or her facility. This supplement will be filed in Academic Affairs and be accessible to key people in the Administration and Public Affairs should an emergency arise. (Your protocol will not be filed in the same location nor will it be accessible to Public Affairs.) Please attach the description to this Protocol form. The description should cover:**

**Protocol Title:**

- a) **Purpose of the project (questions it seeks to answer, health problems it addresses, or in the case of basic research, the reasons, in terms of human or animal health and safety, why the research is important)**
  
- b) **Person to be contacted if additional information is necessary, as well as emergency phone numbers**
  
- c) **Species (and number) used and reason for selection**
  
- d) **General procedures involved in the project**
  
- e) **Steps taken to ensure minimal pain and/or distress to animals**
  
- f) **Results achieved to date and any health problems to which they may have been or may be applied, particularly if the project has been going on for a number of years**
  
- g) **Non-Animal methodologies used as adjuncts in the study, if any, or if adjuncts are not used, reasons why they are inappropriate**

**h) Contract testing/research projects and assigned/granted to other institutions, both commercial and academic**

**i) Potential human or animal health dangers if research animals are released**

**Minnesota State University Moorhead  
Institutional Animal Care and Use Committee (IACUC)  
Protocol Amendment Form**

1.     **Date Submitted: (mm/dd/yy)**
  
2.     **Principal Investigator(s):**
  
3.     **Protocol Code:**
  
4.     **Protocol Title:**
  
5.     **USDA Classification:**
  
6.     **Changes to be made:**
  
  
  
  
  
  
  
  
  
  
7.     **Reason for changes:**
  
  
  
  
  
  
  
  
  
  
8.     **Personnel Changes:**

This form should only be used for minor changes such as adding or dropping species, adding or dropping researchers or other similar minor changes to an existing approved protocol (three year approval). For all other changes to an existing approved protocol, complete a new protocol form highlighting the questions where the new procedures will be performed or where existing procedures will be deleted.

Protocol amendment forms must have the signature of the principal investigator/course instructor as well as all other investigators/course instructors listed on the protocol to indicate their awareness of the proposed changes to the protocol.

<b>9.</b>	<b>Signature(s) of Investigator(s)</b>	<b>Date</b>
	_____	_____
	_____	_____
	_____	_____
<b>10.</b>	<b>Approved by IACUC</b>	
	_____	_____

## I. Facility Inspections

The Institutional Animal Care and Use Committee (IACUC) is required to review the institution's program for humane care and use of animals at least once every six months. This review includes an inspection of all animal care and use facilities within the institution. The Guide for the Care and Use of Laboratory Animals (2002) is used as a basis for evaluation. All the animal housing, laboratories, surgery areas, etc., where animals are used, must be inspected. This inspection is made by members and representatives of the IACUC, and the report of the committee is submitted to the responsible Institutional Official. Significant and minor deficiencies are differentiated and plans to correct these deficiencies detailed. These review and inspection reports are kept on record and made available to APHIS and OLAW on request.

## J. "Verification" in the Responsible Use and Care of Animals in Research and Teaching

The 1986 Amendment of the Animal Welfare Act, the PHS Policy, the NIH Guide, and MSUM Policy all require that personnel involved with laboratory animal care and use be properly trained and aware of laws and regulations concerning animal use. MSUM established a policy in 1988 that all personnel involved with animal care and use should have a basic knowledge and understanding of the principles of humane and effective use of laboratory animals, and of the obligations incumbent on those who utilize those animals. **The MSUM Handbook for the Responsible Care and Use of Animals in Research and Teaching** (Rev. 1997 & 2009) **is the key reference for obtaining MSUM verification in Responsible Use and Care of Animals in Research and Teaching. The Basic Principles of Laboratory Animal Care in Part V of this document must be studied and the exam must be completed by all personnel who have not previously been certified. Animal Use Protocol Reviews will not be reviewed unless all personnel are certified. Updating will be required every five years.** Copies of the IACUC questionnaire used to obtain verification are available through the IACUC chairperson.

## K. Animal Activity Forms for USDA

Acquisition records, information on the numbers and species of animals used and the invasiveness of the procedure are required in order to complete the annual institutional USDA report. The report period is from October 1 of the preceding year to September 30 of the current year. **This report must be received by the IACUC no later than November 1.** Good inventory records kept by each investigator/educator are required to generate this annual report. Pain classifications should be listed according to the scheme detailed under No. 2, Category Research of the MSUM Request for Review of Research/Biological Testing Protocol.

## L. Infractions

In the event that an infraction of the animal care and use regulations is observed or mistreatment of a research/teaching animal should occur or be suspected, it is the responsibility of the observer to report this to the principal investigator and/or the department chair. In the event that this is not possible, or no corrective action is taken, the problem should be reported to an IACUC member. The IACUC will investigate the matter and, if the complaint is valid, discuss what steps are needed to correct the situation with the personnel involved. As this can be an extremely serious matter, it is essential not only that the complaint be accurate and just, but that there be no discussion of it except with the appropriate people. While it is important that these events be reported, it is also only fair to ensure the validity of the complaint prior to any outside publication.

## II. Training Opportunities

### A. AWA and PHS Policy on personnel caring for and/or using animals

Both the AWA and the PHS Policy require that personnel caring for and/or using animals be appropriately qualified and trained in those procedures. It is the immediate responsibility of the principal investigator/course instructor to ensure that all his/her personnel are properly trained and supervised with regard to the care and use of laboratory/educational animals. This Handbook is designed to introduce individuals involved with animal use to pertinent regulations, guidelines, ethics, and safety issues. There are several additional ways in which individuals may extend their knowledge in these areas. For example, investigators may avail themselves of other appropriate training such as through the American Association for Laboratory Animal Science (AALAS), 70 Timber Creek Drive, Cordova, TN 38018, (901) 754-8620, AALAS publishes a bi-monthly journal, Laboratory Animal Science, and has developed a certification process for animal caretakers and technicians which has three training manuals and levels: Assistant Laboratory Animal Technician, Laboratory Animal Technician, and Laboratory Animal Technologist.

#### B. Occupational Health Program

The NIH Guide (2002) states that "An occupational health and safety program must be part of the overall animal care and use program". **As stated in Minnesota State University Moorhead's PHS Assurance document, all personnel working with animals should have a current tetanus immunization; should study Module 4, Occupational Health and Safety, of the Canadian Council of Animal Care ([www.ccae.ca/en/CCACMain.htm](http://www.ccae.ca/en/CCACMain.htm)), and answer the questions on the module; and should complete the medical history questionnaire found on the IACUC webpage, [www.mnstate.edu/iacuc](http://www.mnstate.edu/iacuc). The Canadian Council of Animal Care can also be accessed through a link on the IACUC webpage.** People working with nonvaccinated dogs and cats should get a rabies vaccination. All personnel who are enrolled in a health maintenance organization should utilize their physical examination benefit on an annual basis. Students may take some advantage of the Hendrix Health Center.

MSUM has two types of incident report forms. Depending upon the status of the injured party, one of the two forms should be completed. The forms are typically available through the academic department or the MSUM Personnel and Administrative Affairs offices.

### Part 3

#### Husbandry and Management

##### I. Laboratory Animal Health

###### A. Animals lost to disease or trauma

Animals lost to disease or trauma before the conclusion of an experiment are costly and can affect the significance of research results. Good animal health management practices, including proper group housing to optimize breeding and minimize stress and trauma, are essential. Subclinical disease can affect the physiologic function of many bodily systems; good experimental quality control requires careful microbial and serologic surveillance.

###### B. Veterinary care and surveillance

Veterinary care and surveillance are an essential part of the animal care program. The health of new animals is to be carefully ascertained with appropriate physical examination and observation. Laboratory services of the North Dakota State University veterinary diagnostic laboratory may be utilized for surveillance and quality control of new animals. Disease investigation may include parasitic, bacteriological or viral screening. These procedures are appropriately performed during

conditioning or quarantine periods prior to the initiation of the experimental use of the animal.  
**Animals are to be observed daily to assess their health and welfare.**

#### C. Separation of animal species

It is sound policy to separate animals by species, source and health status. Separation by species is required by the Guide (1996). This does not necessarily mean that rodents such as mice and rats may not be housed in the same room. Where space permits, separation by shipment and vendor are practices strongly recommended. Most vendors of laboratory rodents will provide data detailing their own health surveillance on colonies of production animals. A copy of these records should be sent to the IACUC chairperson.

#### D. Good animal health

Maintaining good animal health is dependent on the quality of facilities and care. It also depends on sanitation, pest control and other aspects of facility and housing management. Any problem within your facility or questions about housing should be addressed to the IACUC veterinarian.

## II. Laboratory Animal Facilities

### A. Housing

Housing requirements for laboratory animals are detailed in the Guide (2002), Chapter 2, Animal Environment, Housing and Management. A good husbandry program provides a system of housing and care that permits animals to grow, mature, reproduce, and maintain good health. Good husbandry minimizes variations that can modify an animal's response to experimentation.

### B. Caging or housing systems should: (exerpted from GUIDE, 2002)

1. Allow for the normal physiologic and behavioral needs of the animals, including urination and defecation, maintenance of body temperature, normal movement and postural adjustments, and, where indicated, reproduction.
2. Allow conspecific social interaction and development of hierarchies within or between enclosures.
3. Make it possible for the animals to remain clean and dry (as consistent with the requirements of the species).
4. Allow adequate ventilation.
5. Allow the animals access to food and water and permit easy filling, refilling, changing, servicing, and cleaning of food and water utensils.
6. Provide a secure environment that does not allow escape of or accidental entrapment of animals or their appendages between opposing surfaces or by structural openings.
7. Provide housing and facilities that are free of sharp edges or projections that could cause injury to the animals.
8. Allow observation of the animals with minimal disturbance to them.

### C. Cages

Cages should be constructed to allow for ease of sanitation. Surfaces and angles should be smooth. The design should allow for visual inspection without disturbing occupants. Particular attention is required to keep cages in good repair and to replace those that are deteriorated. Consideration should be given to the social environment. When appropriate, group housing is preferred. It is important to take into account population density, compatibility, age, sex, and social

rank. Group composition should be as stable as possible. Mixing groups or introducing new members can alter behavior, as well as physiological function.

#### IV. Space Recommendation

TABLE 1. Minimum Space Recommendations for Laboratory Animals (excerpted from GUIDE, 2002)

Animals	Type of Weight	Housing	Height		in	cm
			Floor Area/Animal			
	<u>g</u>		<u>in<sup>2</sup></u>	<u>cm<sup>2</sup></u>		
Mice	<10	Cage	6.0	38.71	5	12.70
	10-15	Cage	8.0	51.62	5	12.70
	15-25	Cage	12.0	77.42	5	12.70
	>25	Cage	15.0	96.78	5	12.70
Rats	<100	Cage	17.0	109.68	7	17.78
	100-200	Cage	23.0	148.40	7	17.78
	200-300	Cage	29.0	187.11	7	17.78
	300-400	Cage	40.0	258.08	7	17.78
	400-500	Cage	60.0	387.12	7	17.78
	>500	Cage	70.0	451.64	7	17.78
Hamsters	<60	Cage	10.0	64.52	6	15.24
	60-80	Cage	13.0	83.88	6	15.24
	80-100	Cage	16.0	103.23	6	15.24
	>100	Cage	19.0	122.59	6	15.24
Guinea pigs	350	Cage	60.0	387.12	7	17.78
	>350	Cage	101.0	651.65	7	17.78
	<u>kg</u>		<u>ft<sup>2</sup></u>	<u>m<sup>2</sup></u>		
Rabbits	<2	Cage	1.5	0.14	14	35.56
	2-4	Cage	3.0	0.28	14	35.56
	4.5-4	Cage	4.0	0.37	14	35.56
	>5.4	Cage	5.0	0.46	14	35.56
Pigeons	--	Cage	0.8	0.074	(able to stand erect w/feet on floor)	

## Micro- and Macro-environments

The cage is considered the micro-environment, while the room where the animals are held would constitute the macro-environment. Conditions in the cage (temperature, humidity, ventilation exchange) can be vastly different from that of the room. This is especially true in bonneted or filtered rodent cages. Ammonia build-up is common in such systems if bedding is not frequently changed or air change is deficient. Respiratory infections and distress can then result.

## V. Temperature and Humidity Recommendations

TABLE 2. Recommended Relative Humidity and Dry-Bulb Temperature for Common Laboratory Animals (excerpted from GUIDE, 2002)

Animal	Relative Humidity (%) <sup>a</sup>	Dry-Bulb Temperature <sup>a</sup>	
		°C	°F
Mouse	40-70	18-26	64.4-78.8
Rat	40-70	18-26	64.4-78.8
Hamster	40-70	18-26	64.4-78.8
Guinea pig	40-70	18-26	64.4-78.8
Rabbit	40-60	16-21	60.8-69.8

<sup>a</sup>From ILAR. 1965, 1966, 1973a, 1977, 1978a,b, 1980.

## VI Ventilation

Adequate ventilation of animal rooms is very important to animal health. The Guide recommends at least 10 to 15 air changes per hour in all animal rooms. Quarantine and biohazard containment areas must be maintained under negative pressure relative to the hallway. As a general rule, no animal room should smell excessively of ammonia or feel excessively humid or "stuffy." If you suspect a ventilation problem in an animal room, the IACUC chairperson or department chairperson can help arrange for testing.

## VII Illumination

Proper lighting in animal rooms aids in sanitation. In addition, the amount of light some species receive daily can affect hormone levels, reproduction, and other physiologic variables. The Guide recommends the use of light timers for proper diurnal light cycles in all animal rooms. Intense prolonged light exposure can have a detrimental effect on the retinas of some rodents.

## VIII Feed

Storage areas for feed and bedding must be closely monitored for adequate pest control. All feed and bedding bags should be stored off the floor on shelves or pallets. Feed should be used within 90 days of receipt, as some nutrients deteriorate under long-term storage. **Feed must be stored in a closed container after a feed bag has been opened.**

## **IX Bedding**

Bedding should be free of toxic chemicals or contaminants. Aromatic hydrocarbons from cedar and pine materials can induce biosynthesis of hepatic microsomal enzymes, thus altering some experiments.

## **X Water**

Continuous access to fresh, potable, uncontaminated drinking water is required. Water quality should be periodically monitored. It may be desirable to provide acidified water to rodents that are compromised or otherwise stressed. This will help prevent pseudomonas infections in such animals. (See your veterinarian for details.) Watering devices, sipper tubes, and automatic waterers should be routinely examined for proper function.

## **XI Sanitation**

### **A. Animal housing areas**

Sanitation is essential in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be cleaned with appropriate detergents or disinfectants as often as necessary. Cleaning utensils, mops, pails, and brooms should not be transported between animal rooms.

### **B. Bedding**

Bedding for larger species should be changed daily; for rodents, one to three times weekly. If animal waste is removed by hosing or flushing, this should be done at least once daily. Litter from cages should be emptied in an area other than the animal room. Where pheromones are essential to reproduction, cage cleaning may be less frequent. Cages are to be washed and sanitized at least every two weeks. Disinfection is achieved at 180<sup>o</sup> F. Autoclaving of sipper tubes is recommended.

### **C. Deodorizers**

Deodorizers are not appropriate to most animal odors. Such products are not a substitute for good sanitation and can also cause change in microsomal enzymes.

## **XII. Important Zoonoses in Laboratory Animals.**

### **A. Zoonotic pathogens**

Various zoonotic pathogens are transmissible from animals to humans. It is the responsibility of the researcher and assistants to become familiar with those diseases which apply to the animals used, their transmission and safety measures. The GUIDE, 2002, specifically states that those conducting field investigations should assure IACUC that their procedures comply with State and Federal regulations. Zoonoses and occupational health issues should be reviewed by IACUC to insure that the safety and health of humans or other animals are not compromised.

### **B. Quality control programs**

Conventional quality control programs may not be completely suitable for some wildlife species. These animals should never be brought into research laboratories and housed with existing colonies without the benefit of a quarantine period (at least two weeks). Expert advice should be sought on testing for disease problems, handling procedures and potential zoonotic risks whenever a new species is brought in. All such animals should be handled with gloves and protective clothing. The National Wildlife Lab in Madison, WI, can be an additional source of information on disease and special considerations in using wild species.

Certain wildlife diseases are recognized as having serious zoonotic potential to humans. Parasites such as Baylisascaris procyonis, which is found in raccoons, can cause serious brain and eye infections by migrations of the larval stages. Salmonella and Arizona sp. in reptiles (and many wild

mammals) can cause serious diarrhea in both animals and man. Recently, wild caught Peromyscus were found to be infected with the spirochete Borrelia burgdorferi, the causative organism of Lyme disease. The organism can be spread by urine and oral transmission. In the wild, both man and animals are usually infected by ticks carrying the spirochete. The tick bite generally causes a skin rash, but cardiac abnormalities, neurologic signs, and degenerative arthritis are the sequelae of any route of infection.

C. The Guide, 2002

The GUIDE, 2002, also specifies that high standards of cleanliness be maintained and that appropriate clothing and protective gear be worn. Food and drink are not permitted in animal rooms.

Please study the GUIDE, 2002, for more information.

**XIII. General Principles for Minimizing Risk**

- A. Do not eat, drink or smoke in animal rooms or procedure rooms. Contact between the hands and the nose, eyes, or mouth must be avoided.
- B. Wear disposable gloves when handling animals, bedding, or soiled cages.
- C. Protect clothing with outer disposable or washable garments (lab coat, gown, coverall).
- D. Exercise great care with needles. Keep needle capped until ready to use. Dispose of used needles properly and promptly.
- E. Bites and other wounds should be washed immediately and followed by appropriate medical attention.
- F. Hands should be washed with soap and water immediately after handling all animals and animal equipment, and before leaving the laboratory or animal facility.

**XIV. Biological and Radiation Hazards**

A. Biohazards

Projects involving infectious agents potentially hazardous to humans are an important part of biomedical research. When working with such agents, maintenance of a healthy, uncontaminated laboratory environment is of paramount importance. No procedure is so urgent that time considerations override safety.

B. Radiation Hazards

Contact the Radio-Isotope Committee for more information.

## **Part 4**

### **APPENDICES**

#### **APPENDIX A**

##### **General References**

Guide for the Care and Use of Laboratory Animals, DHHS (NIH), Revised, 1996. National Academy Press, Washington, D.C. ISBN No. 0-309-05377-3.

Animal and Plant Health Inspection Service, 1995, U.S. Government Printing Office, U.S. Department of Agriculture, Washington, DC 20250.

Public Health Service Policy on Humane Care and Use of Laboratory Animals, OPRR at National Institutes of Health, 6100 Executive Blvd., MSC 7507, Suite 3301, Rockville, MD. 20892-7507

For additional readings, references and agencies the reader is referred to the GUIDE, 2002, Appendices A-D.

## APPENDIX B

### EUTHANASIA GUIDELINES

Generally, it is the policy at Minnesota State University Moorhead, USDA, and NIH to follow the guidelines of the AVMA Panel on Euthanasia. The most recent edition was published in 2001; copies are available from the IACUC chair. Modes, methods, agents, and precautions are discussed in the report. All vertebrate animals on campus that are to be killed during the course of or at the conclusion of an experiment must be euthanized using an approved method. Question #24 and 25 on the MSU protocol form asks for the method of euthanasia. Euthanasia by definition is a quick, painless death. Pain can be defined as the perception that results from nerve impulses reaching the cerebral cortex. Therefore, the unconscious animal cannot experience pain. Rapidly occurring unconsciousness followed by respiratory and cardiac arrest is required for the method to be acceptable. At the conclusion of the euthanasia procedure, a follow-up exam is required to confirm the absence of a heartbeat. There are horror stories of animals assumed to be dead who reportedly begin to revive later.

The investigator has the responsibility of choosing a proper euthanasia method both on ethical grounds and on the compatibility with the experimental data needed from postmortem examinations. The humanitarian requirements of the method cannot be ignored in the urgency and/or need to obtain significant experimental results.

Euthanasia may be required during the course of an experiment. Investigators have a moral obligation to alleviate unnecessary pain and suffering. "An animal that is observed to be in a state of severe pain which cannot be alleviated should be immediately destroyed."<sup>1</sup> It is sometimes difficult to recognize pain and suffering and species may vary in their manifestations. All personnel working with animals should be familiar with the signs of pain and suffering in their particular species. Clinically obvious depression, loss of appetite and vocalization are possible signs of stress and pain.

The following section on Euthanasia was excerpted from the GUIDE, 2002.

Euthanasia is the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the 2001 Report of the AVMA Panel on Euthanasia (AVMA 2001 or later editions). In evaluating the appropriateness of methods, some of the criteria that should be considered are ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; nonreversibility; time required to induce unconsciousness; species and age limitations; compatibility with research objectives; and safety of and emotional effect on personnel.

Euthanasia might be necessary at the end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments. Protocols should include criteria for initiating euthanasia, such as degree of a physical or behavioral deficit or tumor size, that will enable a prompt decision to be made by the veterinarian and the investigator to ensure that the end point is humane and the objective of the protocol is achieved.

Euthanasia should be carried out in a manner that avoids animal distress. In some cases, vocalization and release of pheromones occur during induction of unconsciousness. For that reason, other animals should not be present when euthanasia is performed (AVMA 2001).

The selection of specific agents and methods for euthanasia will depend on the species involved and the objectives of the protocol. Generally, inhalant or noninhalant chemical agents (such as barbiturates, nonexplosive inhalant anesthetics, and CO<sub>2</sub>) are preferable to physical methods (such as cervical dislocation, decapitation, and use of a penetrating captive bolt). However, scientific considerations might preclude the use of chemical agents for some protocols. All methods of euthanasia should be reviewed and approved by the IACUC.

It is essential that euthanasia be performed by personnel who are skilled in methods for the species in question and that it be performed in a professional and compassionate manner. Death should be confirmed by personnel who can recognize cessation of vital signs in the species being euthanized. Euthanizing animals is psychologically difficult for some animal-care, veterinary, and research personnel, particularly if they are involved in performing euthanasia repetitively or if they have become emotionally

attached to the animals being euthanatized (Arluke 1990; NRC 1992; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be aware of this as a potential problem for some employees or students.

### MODES AND METHODS

Judging the humaneness of a particular euthanasia method can be extremely difficult for an uninformed observer. During the process of euthanasia, movements of an animal's body (muscle contractions) do not necessarily indicate consciousness, nor does lack of movement in response to painful stimuli indicate the absence of pain (such as under the influence of curariform drugs)!

There are three modes of action for euthanizing agents.

1. Hypoxia/hypoxemia
2. Direct depression of neurons
3. Physical damage to brain (CNS) tissue

The best choice depends on the use of the animal postmortem, safety to personnel, and the ethical considerations already discussed.

### METHODS

As in any procedure involving animals, restraint and handling play a vital role. The proper physical control and environment will minimize discomfort and maximize safety and smoothness of operation. The procedure should be performed out of sight and hearing range of other animals and in a calm and preferably familiar environment.

#### Unacceptable methods:

1. Strychnine
2. Curariform drugs alone are condemned
3. Exsanguination without prior anesthesia
4. Drowning without prior anesthesia
5. Electrocution is strictly forbidden unless the current passes directly through the brain and the safety and effectiveness of the technique can be demonstrated to an RARC veterinarian.

### RECOMMENDED METHODS BY ANIMAL GROUPS

#### RODENTS:

1. Inhalation agent overdose (ether, halothane, methoxyflurane). These must be used under a hood. They act by direct depression of vital neurons. This is one of the less desirable methods due to its danger to personnel and difficulty in volatilizing methoxyflurane.
2. Guillotine (decapitation). This is to be used with small rodents only. Some recommend that the head be immediately placed in liquid nitrogen. However, a new study questions the effectiveness of using liquid nitrogen. We now recommend that the animal be sedated or lightly anesthetized. There are few tissue changes with this technique. Since existing data suggests that animals remain conscious for 13-14 seconds following decapitation, we discourage this technique and will accept it only with scientific justification and scrutiny.
3. Nitrogen gas. Although effective in a closed chamber system, it is less preferable than other methods. Newborn animals will survive much longer than adults. Rats may exhibit signs of distress and panic before collapsing and dying. This is a hypoxic agent; tissue changes associated with hypoxemia may occur.

4. CO<sub>2</sub> chamber. CO<sub>2</sub> rapidly acts by direct depression of cerebral cortex. Length of time to effect may be prolonged in immature animals. There are no changes in cellular structure, however, some involuntary muscle activity after unconsciousness may be noticed. There are specific chambers made for this purpose. It is important that the flow be adequate to eliminate layering of the gas which will cause the animals to seek any air left at the top of the chamber thus causing death from stress and not from hypoxia. This is the preferred method under the proper conditions.
5. Barbiturate overdose. Given intraperitoneally in rodents, this works quickly, as it does in all species, by depressing CNS functioning beginning in the cerebral cortex and moving to the respiratory center. Cardiac arrest quickly follows. Pentobarbital is the most frequently used barbiturate. It will cause drug residues and an enlarged spleen. Aesthetically it is considered more acceptable due to the appearance of putting the animal under general anesthesia first. A disadvantage is that the DEA requires strict accounting of all drug use.
6. Cervical Dislocation. (Used in mice, hamsters, gerbils and immature rats.) This technique causes direct depression of the brain. It may be distasteful to some due to violent muscle contractions which usually follow. The technique requires training and skill. The tissues remain unaltered by chemicals. The best choice for individual animals when the technician has proper training and skills.
7. Microwave. Only microwave chambers specifically designed for use on rats and mice can be used. They direct most of their energy to the head causing immediate unconsciousness and death. The method is useful for neurobiologists in fixing brain metabolites without loss of anatomical integrity.

#### **REPTILES:**

Reptiles will become more tractable if they are refrigerated at 40-45 degrees for 15-20 minutes. Ketamine can be given to induce general anesthesia. At this point decapitation and/or exsanguination is a suitable method for euthanasia. An overdose of pentobarbital or other barbiturate given IP or IV is preferable. Confirmation of death requires more care in reptiles particularly if they have been refrigerated first.

#### **BIRDS:**

Because of the bird's high respiratory rate, an overdose of anesthetic inhalant agents will work well (i.e. ether, halothane, methoxyflurane). Personnel, however, must take precautions when working with these agents. CO<sub>2</sub> also works well, but birds may show some distress before losing consciousness. T-61 appears to have a smoother induction than barbiturates in birds. These are given IV, most often injected in the wing vein. Cervical dislocation is acceptable only in chickens and small turkeys.

#### **RABBITS:**

Putting companion type animals "to sleep" is best accomplished by using barbiturates or specific euthanizing agents such as T-61. These drugs must be given IV; veins in all these species are readily accessible.

1. Barbiturates. Two people should be used to perform the procedure. Tissue changes from these drugs are well known.
2. T-61. This is a non-narcotic mixture of three drugs, which have general anesthetic, curariform, and local anesthetic actions. Currently it is not controlled by the DEA. The mode of action is direct depression of CNS and heart muscle. There will be tissue residues and the drug may cause pulmonary edema and other tissue lesions.

## Part 5

### MSUM IUCAC Basic Principles of Laboratory Animal Care

#### Rats

Numerous species of animals serve as subjects for education demonstration and research. However, the laboratory rat seems to be the most used subject. The rat's small size and excellent health make it a superior choice for experimentation. The following presents information about anatomy, general physiology, general care, and breeding of rats as well as euthanasia techniques used on rats.

#### Anatomy

Many texts of rat anatomy are presently available. These range from early line drawings of gross anatomy and morphology (Wills, 1964; Smith and Calhoun, 1968) to a plate atlas of rat gross anatomy (Olds and Olds, 1979). The latter atlas is a particularly useful atlas of the peripheral anatomy of the rat. In addition, several atlases describe the central nervous system of the rat in stereotaxic coordinates. These include the early line-drawn atlas of deGroot (1959) and the photographically enlarged plates of Konig and Klippel (1963), Pellegrino and Cushman (1967), Skinner (1971) and Thompson (1978).

#### Physiological Data for the Rat

Table 1 presents average values for several physiological variables of the adult rat. These average values are intended as general information about the rat; most important among these are the values for body weight, food intake, sexual maturity and body temperature.

**Table 1. Normative physiological values for the adult rat.**

Adult Weight	
Male	300-400 grams
Female	250-300 grams
Life Span	
Usual	2.5-3 years
Maximum Reported	4 years, 8 months
Surface Area	0.03-0.06 sq. meters
Water Consumption	80-110 ml/kg/day
Food Consumption	100 gm/kg/day
Body Temperature	99.5 degree F (37.5 C)
Puberty	50 +/- 10 days
Breeding Season	None
Gestation	21-23 days
Litter Size	8-14 pups
Birth Weight	5-6 gm
Weaning	21 days
Heart Rate	330-480 beats/mn
Blood Pressure	
Systolic	88-184 mm Hg
Diastolic	58-145 mm Hg
Cardiac Output	50 (10-80) ml/min
Respiration Frequency	85.5 (66-114)/min
Urine	
pH	7.3-8.5
Specific Gravity	1.04-1.07

Table adapted from: H.J. Baker, J.R. Lindsey and S.H. Weisbroth (Eds). (1979) The Laboratory Rat, New York: Academic Press.

## Housing

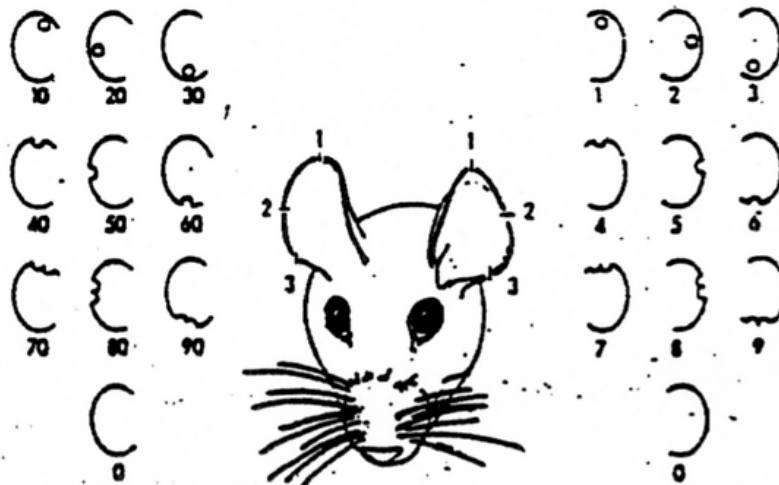
Several organizations have developed explicit guidelines to regulate the care and housing of rodents used in the laboratory (Author, 1978; NIH Guidelines, 1984).. According to the National Academy of Sciences (National Research Council), rats should be housed in spacious, ventilated, and dry cages constructed of either plastic or stainless steel. Bedding (wood shavings, sawdust or commercial litter such as Beta-Chip) should be changed frequently (2-3 times weekly) to minimize odor and to reduce the possibility of disease in the colony. Cages should be sterilized frequently with steam or chemical disinfectants. Humidity within the colony room should be maintained between 40 and 60 percent whereas the temperature should be kept between 70 and 74 degrees F. Lighting should be diffuse throughout the colony and of an intensity (75-125 footcandles) sufficient to allow laboratory procedures to be carried out. Light schedules should be diurnal (i.e. 12 hr/12 hr day/night schedule) because continuous lighting schedules may produce partial retinal degeneration in rats. Tap water and nutritionally complete feed such as Purina Rat & Mouse Diet should be freely available from water bottles and feeders suspended outside of the cage except where inconsistent with the experimental procedures. While it is recognized that not all rat facilities can meet these stringent guidelines, you should be aware of their existence and strive to meet this standard of care.

## Handling

Rodents that are individually housed over a long period frequently display increased emotionality (urination, defecation and, perhaps, aggression) upon handling. Each rat should be weighted to the nearest gram on a balance (scale) daily to assess its general health and to provide daily handling. You should be aware that rapid weight losses (e.g. 10-20 grams overnight) are frequently the first indicator of disease. To remove a rat from a cage, grasp the rat with your thumb and forefinger around the neck preventing biting movements by the rat. If the rat grasps the cage floor with its paws as you attempt to remove it, do not exert excessive pull to remove the rat as a toenail may be torn. Practice picking up the rat, holding it and then replacing it into the home cage. Body handling is the preferred method of handling rats. If tail handling is done, you should pick up the rat by the base of the tail (that part of the tail closest to the body). This method is not recommended for novices, however, as grasping the tip of the tail will frequently shear off the flesh surrounding the tail tip.

## Animal Identification

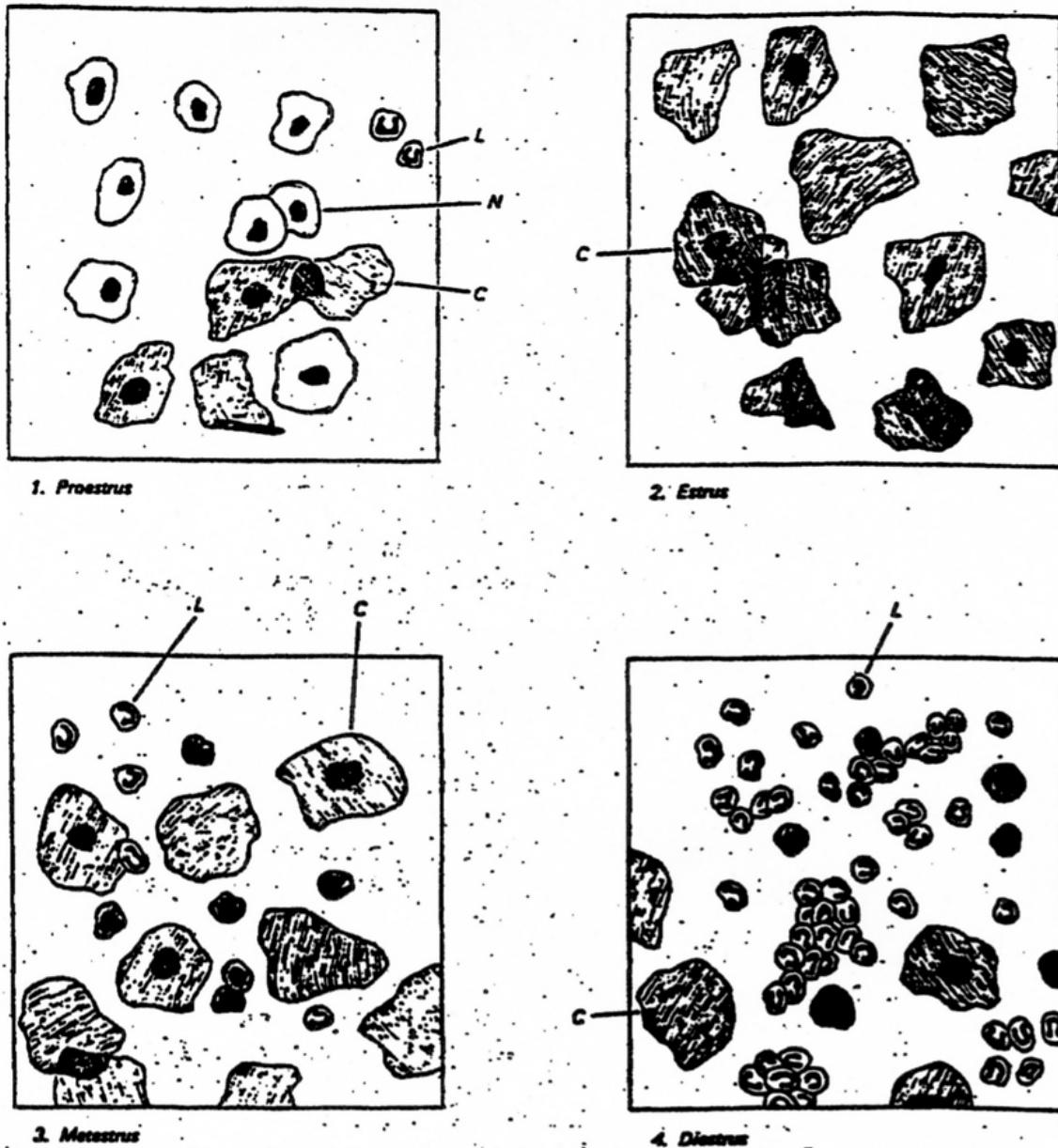
Although rats are frequently group housed for reasons related to available space and expense, experimenters must keep track of individual animal data. Some method, therefore, must be used to identify individual rats. The simplest technique is to place a numbered metal tag in the ear (National Band and Tag Co., 721 York Street, Newport, KY 41071). Other investigators prefer to use an ear notching system in which a punch is used to notch the rats ears using the system illustrated in Figure 1. In this system, one ear is used to represent single digits whereas the other ear is used to represent 10's. A particular number is represented by where on the ear a notch or hole is made. Dyes may be used to identify individual rats. These include India ink on the palmar surface or the inner surface of the ear. In addition, either picric acid (yellow) or carbolfuchsin (red) or an indelible felt-tipped pen can be used to stain the fur on the rat's back. The dyes are typically prepared as 1-5% solutions in 70% alcohol and are applied to the fur using dye-soaked cotton tips.



**Figure 1.** An ear notch system used to identify individual rats. The left ear is notched to represent single digits whereas the right ear is notched to indicate 10's. Adapted with permission from Kraus, A.L. (1980) *Research Methodology*. In: H. J. Baker et al (Eds.), The Laboratory Rat, Volume 2, New York: Academic Press.

### Breeding

The Classic work of Long (1922) described the estrous cycle of the female rat and its clinical characteristics. The rodent estrous cycle is approximately 4 days in length and consists of 4 stages: proestrus, estrus, metestrus and diestrus. Maximal sexual receptivity of the female accompanies estrus, which in the rat occurs 24 hours into the cycle and is indicated by a dry vagina and swollen vulva. Because the estrus cycle is associated with regular changes in the cell type found within the vaginal fluids, cervical smears can be taken daily to estimate the occurrence of estrus. To do so, insert the blunt tip of a disposable pipette containing 1.0 ml of saline into the vagina of the female to be examined. Expel the saline and then a few minutes later, reinsert the pipette and withdraw .25 ml of vaginal fluid. This sample can be smeared on a microscope slide, dipped into 100% alcohol, air-dried and then stained by dipping into a 5% solution of Giemsa stain (Sigma Chemical). Clear the slide by dipping into distilled water, air-dry and then examine the cells on the slide using a light microscope. Using this method, estrus is indicated by the presence of large cornified cells in the vaginal smear (See Figure 2). Other clinical signs of estrus include an ear quiver response induced by stroking the head and back (Farris and Griffith, 1949) or the lordosis response (arched back) induced by manual stimulation of the vulva using a cotton swab.



**Figure 2** Cell types (C, cornified; L, Leutenized) associated with various stages of the estrus cycle. Reprinted with permission from: Adler, N.T. (1976) Induction of pregnancy in female rats by external stimulation. In: Hart, B.J. (Ed) Experimental Psychobiology, San Francisco: W.H. Freeman, Co.

Placement of an adult female rat into a cage with one or more adult male rats for a 6 day period will result in detection of sperm in the vaginal tract and pregnancy (Baker, 1980). Sperm can be detected in the vaginal smear (using the technique described above without staining the vaginal smear) or one can examine the bedding of the rat cage in search of the so-called vaginal plug (a dried mass of sperm and vaginal secretions) that is dislodged from the vagina after

successful copulation. To prevent cannibalism of the offspring by the male, the pregnant female should be isolated in a large cage provided with adequate amounts of food, water and bedding. Gestation in the rat is approximately 21-23 days. The abdomen of a pregnant female rat is distinctly swollen at 13 days of gestation. This is most easily observed by suspending the rat vertically by the tail. Litter size is approximately 8-14. The number of male and female pups are approximately equal in most litters. If the litter size is large (12 or more pups), the litter size should be reduced or culled to 8-10 pups. The pups that are to be euthanized are those that are smallest for their sex. To determine pup sex, one can use the ano-genital distance as an indicator of sex (Myer, 1971). In general, male pups exhibit a larger distance between the anus and the genitals than do females (see Table 2). Moreover, female pups may display rudimentary nipples at about 9-15 days post-partum. Litters should be weaned (i.e. removed from the mother) between 23 and 28 days post-partum with pups placed into either individual or group cages with chow and water freely available. If facilities are not available for breeding, commercial breeders supply rodents of either sex and a given weight range. Moreover, special surgical procedures (i.e. ovariectomy, hypophysectomy etc) are often available from the breeder for a nominal charge.

### Rat Diseases

Great advances in the production of disease-free laboratory rats have been made in the last two decades. Commercial breeders often derive their stock using barrier techniques in which a litter is delivered by caesarean section and raised in a germ free environment. Such rats are remarkably free from diseases of the respiratory and digestive tracts. Upon arrival in the laboratory, however, rats obtained from commercial suppliers should be placed in quarantine for a 7 day period. If skin sores indicating lice or mites are observed, commercially available powders such as Dichlorovose or Equigard (the latter is placed on the cage bedding) will control lice and mites. Viral or mycoplasmal infections may produce upper respiratory difficulties in rats, often indicated by a chattering or wheezing sound. Such infections are highly contagious. Because antibiotic treatments do not readily reverse these conditions, infected animals ( or whole colonies ) should be euthanized (killed) and the colony housing cages should be disinfected with steam or chemical disinfectant. Another common disease observed in rats is labyrinthitis, a bacterial infection of the middle ear. Infected rats display a marked twisting of the body when suspended by the tail. No therapy is available for this disease; infected animals should be euthanized and the colony disinfected. If you are concerned about the health of animals in your care, you should alert your laboratory instructor.

**Table 2. Average ano'genital distance (mm's) in rat pups.**

<u>Age</u>	<u>Male</u>	<u>Female</u>
Newborn	2.8	1.2
7 Days	5.2	2.7
14 Days	8.2	4.9
20 Days	12.0	7.0
42-50 Days	21.0	13.0

Table adapted from: Myers, R.D. (Ed), (1971) Methods in Psychobiology, Volume 1, New York: Academic Press.

### Animal Euthanasia

Care should be taken during the course of an experiment that animals are not

subjected to unnecessary pain or discomfort. Rats that undergo surgical procedures must be rendered incapable of feeling pain via suitable anesthesia. Certainly, the same principle applies to animals that are to be sacrificed or euthanized at the end of a study. The term euthanasia means "good death". Methods of euthanasia should result in a rapid, painless, and humane death for an experimental subject. An accepted technique of euthanasia for rats is lethal injection of pentobarbital (80 mg/ml/kg, intraperitoneal). Such injections rapidly produce unconsciousness and then death. Although this technique may not be suitable for experiments in which biochemical samples are to be collected after death, it is the most humane. Other techniques are described below. They vary in terms of method (physical vs inhalant) and rapidity of death.

### Decapitation

A physical euthanasia procedure is that of decapitation using one of several commercially available guillotines. The rat's head is carefully introduced between the guillotine blades and then separated from the trunk using a rapid movement of the guillotine arm. This procedure may be used to collect large (approximately 5 ml) blood samples or to collect tissue samples in a way that is not compromised by chemically-induced euthanasia. It should be noted, however, that decapitation is not a routine euthanasia technique. Moreover, recent electroencephalic data suggests that decapitation may not produce death as rapidly as once thought (Mlkeska and Klemm, 1975).

### Carbon Dioxide

Placement of rats into a chamber containing highly concentrated carbon dioxide will result in unconsciousness and then death. This technique is often used to euthanize large numbers of rats and is thought to be more humane than decapitation. Inexpensive carbon dioxide euthanasia chambers are described in the literature (Myers, 1971; Kraus, 1980).

### Other Inhalant Gases

Ether, chloroform, halothane and metofane have been used in high concentrations to euthanize rats. Ether and chloroform, although inexpensive, are rather caustic to the lungs of the rat and may result in a painful death. In contrast, halothane and metofane are not caustic, are somewhat rapid but can be prohibitively expensive. Moreover, these gases may be harmful to hepatic function in laboratory personnel that are repeatedly exposed to these gases.

### Carcass Disposal

Death should be verified (absence of heart rate, cool body and rigidity of the body) in any euthanized animal prior to its disposal. Again, if you are in doubt, consult your instructor. Each carcass should be double-wrapped in plastic bags and tagged as to their source. Disposal of carcasses should be carried out according to IACUC recommended practices.

### Mice

Weight: The average mouse weighs less than an ounce (30 grams) and can live up to three years.

Handling: If you are not accustomed to handling mice, it is advisable to wear a snug fitting glove when handling them. To move a mouse, pick it up by its tail with your thumb and first finger grasping it close to the body (if taken by the tip, the whole skin covering may come off). Make sure to leave the mouse in a head down position for only a few seconds, otherwise the mouse may turn, crawl up its own tail, and bite you. To restrain a mouse, grasp by the base of the tail, place it on a surface that it can grip with its fore feet so that you can stretch the body

out, then grasp a fold of the loose skin over the neck and shoulders just tight enough to keep the mouse from turning it's head and biting.

**Housing:** Mice are normally kept in cages measuring six by twelve by six inches high (15x30x15cm) with a solid bottom. The floor is usually covered with one to two inches of woodchips, ground corncobs, sawdust, hay, or any other material available (do not use newsprint, the ink may make the mice sick). The bedding should be changed at least once weekly.

**Feeding:** Mice are nocturnal animals and tend to feed more at nights, however, it is advisable for laboratory animals to have food and water ad libitum (except where experimental procedures dictate otherwise). When using pellets, make sure they small fresh and clean. If they smell musty and moldy, the pellets are old and shouldn't be used. Water bottles should be washed and replaced at least once weekly. Diets can be supplemented with snacks such as cracked corn and/or oil sunflower seeds.

**Breeding:** Female mice are old enough to breed at two months and can have up to seventeen litters a year with an average of five to ten babies per litter. Her reproductive cycle (estrous cycle) makes it possible for her to accept a male every fourth day and so mating occurs often if the male and female are continuously kept in the same cage. More than one female may be kept in one cage with a male (as long as each female has her own nesting box), but it is not recommended to keep two or more males in one cage. When multiple males are housed together they will fight to establish dominance. The Gestation period in mice lasts nineteen to twenty one days. Immediately following birth, babies are tiny, helpless, and hairless weighing approximately one gram. Their eyes are sealed for the first two weeks of life. After they have been weaned, male babies should be placed in separate cages, but female babies can remain together.

**Diseases:**

Bite wounds: Bite wounds will occur between fighting males or overcrowded females. Treat them with a small amount of antibiotic ointment but be careful not to use too much or the mice will eat it when they groom themselves.

Antibiotic Toxicity: Mice are sensitive to streptomycin which can kill them if injected.

Respiratory Infections and Pneumonia: Mice suffering from respiratory infections and pneumonia sit in a corner with fur ruffled up. Discharge from their nose, and chattering noise as they breathe may also be observed. -Antibiotics such as chloramphenicol or tetracycline can be given orally (5mg). twice daily for 4 or 5 days. A 250 mg. capsule of antibiotic can be dissolved in a cupful of water (240cc) and placed in their water bottle. A half teaspoon of sugar can be added to sweeten the water. They should receive 4 to 5 mg of antibiotic daily with this procedure and should be kept on the medication for 5 days.

Diarrhea: Diarrhea may result from improper diet, spoiled food or internal parasites. Replace pellets if they smell musty or smell/look moldy. If there is no improvement in 24 to 48 hours, collect a sample of stool in a plastic vial and have the Vet examine it for internal parasites. If there are parasites the Vet can prescribe the appropriate medication.

Tumor: A tumor is any unusual lump or swelling growing on or in the mouse. Mice are susceptible to tumors and they are common in middle aged and older mice. Some can be removed surgically. Many are malignant and have probably spread extensively before being observed. These mice need to be euthanized to prevent suffering.

Fur chewing: Fur chewing is an indication that the mice are too crowded. Over crowded mice will chew the fur off each others faces. If this occurs they should be placed in separate cages or in larger cages.

Mites, fleas, lice: Mites, fleas, and lice are not commonly seen in mice. If found, their fur should be treated by applying a rotenone-based flea powder weekly. A Shell pest strip containing Vapona may also be placed on top of the cage one night weekly. The strip should not be left on the cage continuously or placed where the mice can chew on it or lick it.

## **Rabbits**

**Weight:** Rabbits range from 2 to 20 pounds and can live for up to 15 years. They are born with closed eyes which do not open until about ten days after birth. Bunnies should be weaned at about 4 weeks old.

**Handling:** To pick up a rabbit, take hold of a handful of loose skin over the shoulders. As you start to lift, slide the other hand under its hind legs and support the body weight by allowing the rabbit to stand on your hand. It is very important that you put your hand under its legs before lifting the rabbit too high. If you fail to do so, it could cause serious damage to the rabbit. The reason for this is if it becomes startled, it will kick its hind legs and possibly break its back. This is particularly true for adult Jack rabbits. Under no circumstances should you grab a rabbit by the ears. It is painful for the rabbit and during the struggle it could damage the fragile blood vessels in the ears. Also, do not grab the rabbit with both hands around its middle. Rabbits do not like this and will try to escape. As they struggle to escape, they may scratch you and can injure their back. In the event that a rabbit is being given sub-cutaneous injections in the back which may produce sores, the animal can be cupped to the chest of the handler using both hands. However, special care has to be taken to prevent the animal from scratching the handler and/or injuring its back if it struggles to escape.

**Housing:** A rabbit must have a hutch or a cage for its own security. An adult rabbit weighing 8 to 12 pounds requires at least 1 square yard (1 square meter) of floor space. A rabbit can tolerate cool much better than hot. The hutch should be protected from the sun and from wind. Bedding should be changed 2-3 times per week.

**Feeding:** Rabbits are herbivorous animals. They eat most types of greens, hay, and vegetables. There are also complete diets available in pellet form. This is the preferred diet in most animal research labs. Fresh, clean water must be available at all times. A rabbit will drink 1-2 ounces of water per pound per day. A salt block or ring can be provided and the rabbit will lick to obtain salt as needed. Rabbits teeth grow continuously so they must chew to keep them down. Providing them with hard wood blocks or dry hay or grass will provide for tooth wearing. Feeding utensils and dishes should be kept clean. Wash as needed but they should be washed at least once weekly. Cages should also be cleaned once weekly. All rabbits eat some of their droppings. In the early morning they produce softer pellets covered with mucus, which they swallow as it is passed from their anus. This is a form of recycling called Coprophagy. Disgusting though it may seem to us, the rabbit obtains vitamins produced by intestinal bacteria and additional protein broken down by these bacteria. Thus Coprophagy serves an important function in rabbit nutrition.

**Diseases:** Singly housed rabbits have few disease problems, however there are a few commonly seen diseases.

Ear Mites: These are small insects that live and reproduce in the ear canal.

Symptoms: The infection will produce a smelly, dark-colored discharge. The ear will droop, and the rabbit will scratch its ear and shake its head often.

Treatment: Rub mineral oil or olive oil on both canals twice a week for a month.

Diarrhea: Overeating of greens the rabbit is not used to can cause diarrhea. Cut back on the amount of greens offered. Virulent infections can also cause diarrhea in young rabbits. This is usually fatal.

Paralysis of the rear legs:

Symptoms: The rabbit is unable to use its hind legs and will drag itself along the floor. This is usually the sequel to improper handling, which resulted in the rabbit tearing muscles in the back or fracturing the back. Place food and water close by where the rabbit can reach them without much movement.

Treatment: If the rabbit does not get better in three weeks from keeping still, euthanasia is the humane course to follow.

Heatstroke:

Symptoms: When it is hot and humid, the rabbit may begin to pant and drool. If it is not cooled down, it will go into a coma.

Treatment: If this happens, set up a hose to spray a fine mist or set a tray of ice in the cage when it is hot and humid to prevent it from happening.

Buck Tooth or Malocclusion:

Symptoms: If the rabbit does not have anything to chew on, its teeth will become long and twisted and the rabbit will not be able to eat. There is also an inherited condition in which the rabbits lower incisors can grow out in front of the uppers. If there is no wear the teeth will grow overly long.

Treatment: Trimming the teeth with a cutting pliers if they get long, or Just providing chewing materials should give relief.

Pregnancy Toxemia:

Symptoms: This is a condition that sometimes appears in overweight does at or shortly after kindling. It can result in death even with veterinary treatment. The doe is found lying comatose in the hutch.

Treatment: You should first take it to the Veterinarian. There it will be treated with 10ml/50 percent glucose solution intravenously. If the vet. is not available, and you are forced to treat the rabbit yourself, give the rabbit a simple sugar solution orally. Honey can usually be used as a simple sugar (5cc honey In 30 ml water). Insert a plastic tube (1/8 or 1/4 inch [3-6mm] by 12 Inches [36cm]) down the rabbits throat in to its stomach. Use a large syringe to inject the liquid in to the tube. If you do not have access to a syringe you can use a plastic food dispenser (ketchup or mustard bottle) or a turkey baster. Lay the doe on her left side with her head pointed to her right. Measure from the rabbits last rib to its nose and mark the distance on the tube. Insert the tube into the rabbits stomach until the mark on the tube reaches the rabbits nose. The tube can be lubricated with margarine or butter to facilitate its insertion. Make sure

to push it gently into the back part of the mouth and if it hits an obstruction, withdraw a little way and start down again until it slides down the throat and the mark on the tube reaches the rabbits nose. If the rabbit is still alive but not up and around in about an hour, repeat the procedure. If it recovers, a solution of one teaspoon of sugar per pint of water should be placed in its waterer for five or six consecutive days.

Upper Respiratory Infection: This is usually a bacterial infection, is frequently caused by a Pasteurella organism and is called snuffles. If left untreated, it can lead to middle ear infection, pneumonia and death.

Symptoms: The symptoms of upper respiratory infections are: sneezing, coughing, and mucus discharge from eyes and nose.

Treatment: This can be effectively treated with 80 mg/kg of an antibiotic/day (Panmycin), which should be divided into several doses over the day.

#### Internal Parasites:

Symptoms: The rabbit can get roundworms, tapeworms, and coccidia.

Treatment: If you see what looks like parasites being passed by a rabbit, collect several samples of the stool in a plastic bottle and take them to the Vet. He or she will identify the parasites and prescribe the appropriate medication.

### **Guide for the ethical treatment of fish, amphibians and reptiles for research purposes**

We generally follow the guidelines developed by consensus among conscientious members of the Animal Behavior Society (published *Animal Behavior*, 1998), the Fisheries Society of the British Isles (published in the *Journal of Fish Biology* 2006) and the American Society of Ichthyologist and Herpetologist ([www.asih.org](http://www.asih.org), 2004).

1. Choice of species and non-animal alternatives
  - a. Investigators should avoid species that are rare, endangered or listed as species of concern unless the study purports to contribute to species conservation.
  - b. Preference should be given to species that are well suited for captivity to minimize stress induced by captivity.
  - c. The species should be well-suited for the study question at hand.
2. The number of individuals.
  - a. The number of animals used should be the minimum that are sufficient to accomplish the stated research goals.
  - b. Animals collected from the wild should not deplete or detrimentally impact natural populations.
3. Procedures
  - a. Ethical treatment of fish, amphibians and reptiles assumes that these animals are sentient and sufficiently complex to be cognizant of pain and suffering. Therefore, researchers should take the same general measures followed to minimize pain and suffering in warm-blooded vertebrates.
  - b. Field studies:

- i. Investigators must always consider and minimize the effect of their study on the natural resident population and the habitat on which it depends. Researchers bear the responsibility of acquiring knowledge of the local habitat and population in order to minimize their effect on the natural population.
  - ii. Trapping: live traps should be checked frequently (“frequently” depends on the biology of the species)
  - iii. Marking and telemetry: ensure that removed tissue does not affect animal’s survival and general killing:
- c. Aggression, predation and intraspecific killing:
  - i. Reasonable measures should be taken to minimize harm to study animals, including the use of models in lieu of staged encounters with predators, and pre-determined end points.
- d. Aversive stimuli, deprivation and motivation:
  - i. Care should be exercised to ensure that levels of manipulation are no greater than necessary to produce the desired effect.
- e. Social isolation or crowding:
  - i. An understanding of the natural behavior of an animal is necessary to minimize these sources of stress. Some species are stressed by crowding while others are stressed by social isolation.
- f. Deleterious conditions:
  - i. Inducement of disease, increase in parasite load, exposure of animals to pesticides and homeostatic stressors should address possible treatment or alleviation of the condition induced.
- 4. Housing needs vary for each species. The investigator bears the responsibility to ensure adequate space, shelter, food and water, photoperiod, temperature and hygienic living conditions for their study animals. There is not usually any reason to house each species in separate rooms.
- 5. Safety: all facilities housing aquatic animals should be equipped with ground fault interrupter circuits to protect against electrical shock.
- 6. Water quality assurance: the single most important parameter to the health and welfare of fish and amphibians is proper care of water quality.
  - a. Dechlorination: city tap water contains chorine and chloramines. These are both highly toxic to fish and must be removed using either a dechlorinating agent or a filter (e.g. charcoal tower.)
  - b. Filtration: water filtration can be achieved through a variety of means. There are two main types of filtration: mechanical and biological. Coarse media such as sponges of polyester cartridges mechanically remove suspended particles from the water. Filtration media become clogged with debris and must be inspected regularly and cleaned and/or replaced as necessary. Undergravel filters, sponge filters and trickle filters create large surface area for *Nitrosomonas* and *Nitrobacter* bacteria to colonize. These bacteria biologically oxidize ammonia (highly toxic) to nitrite (highly toxic) to nitrate (low toxicity). Regular water changes remove nitrate and prevent it from accumulating.
  - c. Water replacement: The general rule of thumb for water replacement is 10% replacement per week. A gravel vacuum should be used to remove feces and uneaten food from the tank floor

- when siphoning out water during a water change. Turtles and *Xenopus* may require more frequent water changes depending on stocking density and tank volume.
- d. Temperature: Depending on the temperature range of the species, heaters or chillers may be required.
7. Feeding: regular feeding
    - a. Adequate amount: poikilotherms eat small amounts of food. Excess food fouls the water and clogs the filtration system.
    - b. Adequate frequency: for many species, one feeding per day is adequate. More than three feedings per day runs the risk of over-feeding and should usually be limited to larvae.
    - c. Appropriate food type: most fish eat generic flake food, but wild fishes in particular (e.g. stickleback, darters, mudminnows, etc) refuse flakes and must be fed specialized food (e.g. brine shrimp)
  8. Holding conditions
    - a. Appropriate density: crowding increases stress, suppresses the immune system and increases the rate of transmission of parasites and pathogens.
    - b. Appropriate availability of refuge: if aggressive individuals are housed together then refuge is required to protect the subordinate individuals from injury or death.
    - c. Lighting: fish should not be exposed to constant light or constant dark. A 24-hour L:D cycle of 12:12 to 6:18 should be maintained by use of automatic timers.
    - d. Algae: algae is a natural and beneficial part of the natural environment. Algae should be scraped clean of the front viewing pane so that people can easily view the fish and monitor their health and welfare.
  9. Disease Control
    - a. Hygiene: regular water changes (10 % per week for fish) keeps animals stress low and keeps pathogens at low density
    - b. Use of quarantine tank for newly arrived organisms
    - c. An antiseptic net dip to prevent transfer of disease organisms among tank
    - d. When any of these signs develop: inactivity, loss of appetite, drooping fins, labored swimming, labored ventilation, bloat, red-lined rays in the fins, exophthalmia, fungal hyphae on mouth or body surface:
    - e. Remove and euthanize the affected individual by cervical dislocation, or overdose by methanetracaine sulphate (MS222)
    - f. Increase the frequency and proportion of water changes to 50% of tank volume per day until health of the tank's inhabitants stabilizes

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**Part 6**

**IACUC Exam**

Revised April 2009

**MINNESOTA STATE UNIVERSITY MOORHEAD IACUC**

**RESPONSIBLE RESEARCH ANIMAL CARE AND USE EXAMINATION**

Name: \_\_\_\_\_ Department: \_\_\_\_\_

Address (home): \_\_\_\_\_

Social Security #: \_\_\_\_\_ Campus Phone: \_\_\_\_\_

Principal Investigator/Supervisor: \_\_\_\_\_

QUESTIONS: Circle the correct answer. There should be only one correct answer for each item. You can use the training material to assist you in making the correct selection. This exam is designed to “document” your training rather than as a test of your knowledge.

1. The Institutional Animal Care and Use Committee at each institution has the responsibility for:
  - a. choosing the project a faculty member works on.
  - b. providing an Assurance Statement to the USDA.
  - c. insuring the use of alternatives to animals in research on campus.
  - d. insuring that all research animals are provided for and used in accordance with the Animal Welfare act and other pertinent regulations.
  
2. Each institution must provide an ASSURANCE statement to \_\_\_\_\_ in order to be awarded federal funds to conduct research with animals.
  - a. State Department of Agriculture
  - b. USDA
  - c. NIH (OPRR)
  - d. FDA
  
3. The University and NIH policy and “guide” require that warm-blooded animals be observed:
  - a. every 6 hours.
  - b. every 36 hours.
  - c. every 24 hours.
  - d. every 48 hours.
  
4. According to the NIH Principles for Animal Use, experiments involving live vertebrate animals and the procurement of tissues from live animals for research must be performed by or under the supervision of a:
  - a. USDA official.
  - b. NIH qualified technician.
  - c. qualified biological, behavioral, or medical scientist.
  - d. Veterinarian.
  
5. According to the NIH Principles for Animal Use, research projects using animals should be such as to yield:
  - a. significant data.
  - b. fruitful results for the good of society and not random or unnecessary in nature.

- c. results important from an intellectual curiosity viewpoint only.
  - d. results that will contribute to the use of alternatives in research.
6. According to the NIH Principles for Animal Use, \_\_\_\_\_ should be used when appropriate to complement animal experiments and to reduce the numbers of animals used.
    - a. human subjects.
    - b. in vitro biological systems.
    - c. in vivo biological systems.
    - d. speculation
  7. In the event that an experiment protocol would require the death of an animal as an end point, and that this would result in distinct signs of pain and distress in that animal, what should the investigator do?
    - a. Don't do the experiment.
    - b. Finish the experiment quickly and then euthanize the animal.
    - c. Nothing, as the only end point is death.
    - d. Seek an alternative end point.
  8. An organization that accredits laboratory animal facilities is:
    - a. ACLAM.
    - b. AAALAC.
    - c. AHA.
    - d. ASLAP.
  9. Ventilation in animal rooms should provide for how many changes of air per hour?
    - a. 6-7
    - b. 16-18
    - c. 10-15
    - d. 20-22
  10. All food used for research animals must be:
    - a. bought in heavy paper bags.
    - b. specifically mixed for your facility.
    - c. kept in closed containers after opening.
    - d. supplemented with extra vitamin C.
  11. Different species of research animals (with the exception of rats and mice) should not be:
    - a. used in the same experiment.
    - b. housed in the same room.
    - c. fed at the same time.
    - d. used by any one investigator.
  12. Multiple surgical procedures in a single animal:
    - a. are encouraged always.
    - b. are never permitted.
    - c. can be done if you want to.
    - d. can be done if there are sufficient grounds and they are permitted by institutional animal care policies.
  13. After bringing a new animal into your research facility, you should:
    - a. put it immediately in with the others of its species for company.
    - b. hold food and water for 24 hours.
    - c. have it bathed.

- d. consider a period of quarantine and conditioning.
14. The responsibility for enforcing the Animal Welfare Act lies with:
- a. NIH.
  - b. USDA.
  - c. State Department of Agriculture.
  - d. FDA.
15. If left untreated, Snuffles, an upper respiratory infection in rabbits that produces symptoms of sneezing, coughing and mucus discharge from the nose and eyes, will do which of the following?
- a. Make the rabbit resistant to future respiratory infections.
  - b. Go away eventually.
  - c. Make the rabbit infertile.
  - d. Result in death.
16. The proper procedure for reporting alleged violations of the Animal Welfare Act, NIH Principles or guidelines is:
- a. contact NIH and file a complaint.
  - b. call the local newspaper.
  - c. submit a written complaint to IACUC immediately.
  - d. report first to your supervisor, then to the primary investigator, departmental chairman, and finally IACUC should you not get a response before.
17. All persons working with animals should have a current \_\_\_\_\_ immunization.
- a. typhoid.
  - b. diphtheria.
  - c. tetanus.
  - d. yellow fever.
18. If a rat is observed leaning its head to one side and showing marked twisting of its body when suspended by its tail, it should be euthanized and the colony housing should be steam cleaned or chemically disinfected because the rat is suffering from a contagious bacterial infection called:
- a. toxoplasmosis.
  - b. upper respiratory distress.
  - c. labyrinthitis.
  - d. rabies.
19. How often are the animal care facilities required to be inspected by the IACUC?
- a. once a year
  - b. once a quarter
  - c. once every six months
  - d. daily
20. Who is directly responsible for ensuring that personnel working with laboratory animals are properly trained?
- a. Institutional Animal Care and Use Committee
  - b. Department Chairperson
  - c. Dean of Social and Natural Sciences
  - d. Principal Investigator or Course Director/Instructor

21. How often should the bedding in rabbit cages be changed?
  - a. daily
  - b. at least 2 to 3 times per week
  - c. once per week
  - d. 2 to 3 times per month
  
22. Rats are healthiest and most content when kept under
  - a. 24 hour lighting
  - b. 24 hour darkness
  - c. 12 hours of light, 12 hours of dark
  - d. None of the above
  
23. When comparing adult male and female rats of the same strain they are
  - a. About equal in weight
  - b. Males are heavier than females
  - c. Females are heavier than males
  - d. Females are twice as heavy as males
  
24. The safest and most humane way to pick up a rabbit is:
  - a. by the ears
  - b. with both hands just behind the front legs
  - c. with both hands around the middle of the body
  - d. by taking hold of loose skin over the shoulders and supporting the back legs in your other hand
  
25. The gestation period for both mice and rats is approximately:
  - a. Three weeks
  - b. Three months
  - c. Three days
  - d. Three years
  
26. Young mice and rats can be sexed by noting the relative distance between the anus and the genitals.
  - a. Females have a shorter distance between the anus and genitals
  - b. Males have a shorter distance between the anus and genitals
  - c. Females have a longer distance between the anus and genitals
  - d. Females have their anus and genitals overlapping each other
  
27. IACUC (Institutional Animal Care and Use Committee) membership, by federal regulation must include:
  - a. a veterinarian
  - b. an animal researcher
  - c. someone not affiliated with the institution
  - d. someone who does not do animal research
  - e. all of the above are correct.
  
28. If mice housed together are seen chewing their fur this is an indication
  - a. that they are overcrowded
  - b. that they are suffering from mites, fleas and/or lice
  - c. that they are deficient in calcium
  - d. that they are deficient in oils and other nutrients found in their fur.

29. According to NIH Guidelines (1984) rats should be housed in spacious, well ventilated and dry cages constructed from:
- plastic.
  - stainless steel.
  - wood.
  - a or b is correct.
30. Acceptable labeling techniques to identify individual rats include:
- a metal tag in the ear.
  - ear notching.
  - the use of dyes.
  - all of the above.
31. The abdomen of a pregnant female rat is distinctly swollen at 13 days of gestation. This is most easily observed by:
- suspending the rat vertically by the tail.
  - placing the rat on its back.
  - placing the rat on its side.
  - placing the rat on its feet and pulling on its tail while holding it firmly by the shoulders.
32. Which of the following is not true
- An adult rabbit (8lbs.-12lbs.) requires at least one square yard (1 sq meter) of floor space.
  - Rabbits can tolerate cold much better than they can heat thus their cages should be protected from the sun.
  - All rabbits eat some of their droppings. This serves an important function in their nutrition.
  - Rabbits are born with their eyes opened.
33. If rabbits are allowed to overeat on greens that they are not accustomed to they can have:
- Ear mites.
  - Diarrhea.
  - Pregnancy toxemia.
  - Respiratory infections.
34. Buck tooth or malocclusion occurs in rabbits if they are:
- not fed green leafy vegetables.
  - not given salt blocks to lick.
  - not given anything to chew on.
  - all of the above.
35. Female mice are old enough to breed at:
- two months.
  - two weeks.
  - two years.
  - six months.
36. An average mouse weighs less than an ounce (30 grams) and can live up to:
- one year.
  - nine months.
  - ten years.
  - three years.

37. Fighting male mice and overcrowded female mice will bite each other and such wounds can be treated with a small amount of antibiotic ointment. However, if they are injected with the antibiotic streptomycin,
- It will heal their wounds faster.
  - It will prevent them from inflicting bite wounds on each other.
  - It will kill them.
  - It will make them sexually aggressive.
38. Mice can develop diarrhea from:
- improper diet.
  - spoiled food.
  - internal parasites.
  - all of the above.
39. If a mouse is seen sitting in a corner with ungroomed fur, nasal discharge and making chattering noises, he/she most likely has:
- respiratory infection.
  - pneumonia.
  - both a and b.
  - neither of the above. He/she could simply be depressed.
40. If a rat in a colony develops bad upper respiratory disease, the best treatment to prevent the other animals in the colony from developing the disease is to:
- place antibiotics in the water of all of the rats.
  - inject all of the rats with streptomycin.
  - turn up the temperature in the colony and they will sweat out the infection.
  - euthanize the infected rat and steam or chemically disinfect the colony cages.
41. What is the recommended practice to ensure high water quality in lab aquaria?
- 50% water exchange daily
  - 10% water exchange daily
  - 50% water exchange weekly
  - 10% water exchange weekly
  - Top up evaporation weekly
42. Algae growing on the glass is a sign of poor water quality
- True
  - False
43. To minimize the possibility of electric shock, all electrical appliances in an aquarium room (pumps, filters, heaters, lights, etc) should be connected to
- A very long extension cord
  - A battery
  - A back-up generator in case of a power outage
  - A ground-fault interrupter circuit
  - A grounded outlet
44. Daily visual checks of all fish and amphibia are conducted to:
- Check on proper functioning of filtration
  - Check on deportment and general health of animals
  - Rescue victims of aggression
  - Check on water quality
  - All of the above

45. When diseased aquatic animals are discovered, the best response is to:
  - a. Isolate and treat the infected animal in quarantine
  - b. Euthanize and dispose of the diseased animal
  - c. Increase the frequency and percent volume of water exchanges
  - d. Any of the above, depending on the nature of the ailment
  
46. What is the best source of water for housing aquatic animals?
  - a. Unaltered tap water from the municipal supply
  - b. Dechlorinated tap water from the municipal supply
  - c. Deionized water
  - d. Distilled water
  
47. What role do the bacteria *Nitrosomonas* and *Nitrobacter* play?
  - a. They are important disease organisms
  - b. They oxidize ammonia to nitrite and nitrate
  - c. They respond to photoperiod
  - d. Build up to toxic levels when fish are held in crowded conditions
  
48. What use is a gravel vacuum?
  - a. For removing imbedded feces and uneaten food from gravel
  - b. For removing gravel from a clogged drain
  - c. For removing gravel from a tank without removing the water
  - d. A vacuum that uses gravel as a filter medium
  
49. Photoperiod:
  - a. Lights should be on all the time
  - b. Lights should be on only when fish are being observed to reduce stress
  - c. Lights should be on a timer for a 12:12 or ranging from 6:18 to 18:6, L:D.
  - d. Lighting is not important because fish do not need sleep
  
50. Holding animals at high density causes:
  - a. Increased stress
  - b. Decreased water quality
  - c. Increased rate of transmission of disease pathogens
  - d. Increased aggression
  - e. All of the above

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