

The Use of Animals in Biomedical Research (Level II Training)

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Introduction

Why is “The Use of Animals in Biomedical Research” a topic worth presenting?

- Animals continue to play a prominent role in scientific investigations.
- The use of animals is controversial.
- The public’s perception of the use of research animals is often skewed.
- The use of animals has therefore become highly regulated.

An “Animal Care and Use Program” provides mechanisms of institutional oversight to ensure that, in accordance with the public’s expectations, studies are justified and performed using methods that minimize the potential for animal pain and distress.

Required Training (see IACUC Policy at IACUC web-site)

Level I = training brochures, “The Use of Animals in Biomedical Research” and “Introduction to Occupational Hazards Associated with the Care and Use of Laboratory Animals.” Does not allow unescorted access into animal facilities.

Level II = two-hour didactic seminar; required for all personnel wishing to work with laboratory animals and for access into animal facilities.

Level III = “hands-on” one-on-one or group sessions for practical training with animals; may be tailored to the needs of the individual or laboratory.

IACUC Participant Training Log = official record of each individual’s experience and training pertaining to all animal-related procedures s/he is expected to perform; must be updated as needed, available to IACUC/RRF personnel, and *printed prior to semi-annual laboratory inspections* (see Information Sheet at IACUC web-page).

Regulatory Overview

“The Three R’s”

Russell, WMS, and RL Burch. 1959. The Principles of Humane Experimental Technique.

Part One: The Scope of Humane Technique

1. Introduction
2. The Concept of Inhumanity
3. The Ecology of Experimental Animals
4. The Sources, Incidence, and Removal of Inhumanity

Part Two: The Progress of Humane Technique

5. **Replacement** – the ethical obligation to use non-live-animal models whenever possible
 - Comparative Substitution
 - Modes of Absolute and Relative Replacement

- The Principles of Replacement
- The Use of Tissue Culture
- The Use of Micro-organisms

6. **Reduction** - ethical obligation to use only the number of animals necessary and avoid unnecessary duplication

- Reduction and Strategy in Research
- The Problem of Variance
- The Design and Analysis of Experiments
- The Sources of Physiological Variance
- The Control of Phenotype
- The Control of the Proximate, especially Behavioural Environment; Dramatic Variance and Specific Conditions; The Behavioural Environment and Physiological Responses; Towards a New Bioassay

7. **Refinement** – ethical obligation to improve the acquisition, housing, care, and procedure used with animals to reduce variability and any potential pain and/or distress

- Neutral and Stressful Studies
- Generally Superimposed Procedures
- The Choice of Procedures
- The Choice of Species
- A Concrete Problem: Experimental Psychiatry and the Humane Study of Fear; Experimental Psychiatry and the Screening of Tranquilizers; The Use of Lower Species

8. The Factors Governing Progress



Interagency Research Animal Committee (IRAC). 1984. *U.S. Government Principles for the Utilization and Care of Vertebrate Animal Used in Testing, Research, and Training.*

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:

- I. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et seq.) and other applicable Federal laws, guidelines, and policies.*
- II. 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.
- III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.
- IV. Proper use of animals, including the avoidance or minimization 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.

V. 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.

VI. 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 appropriate, during the procedure.

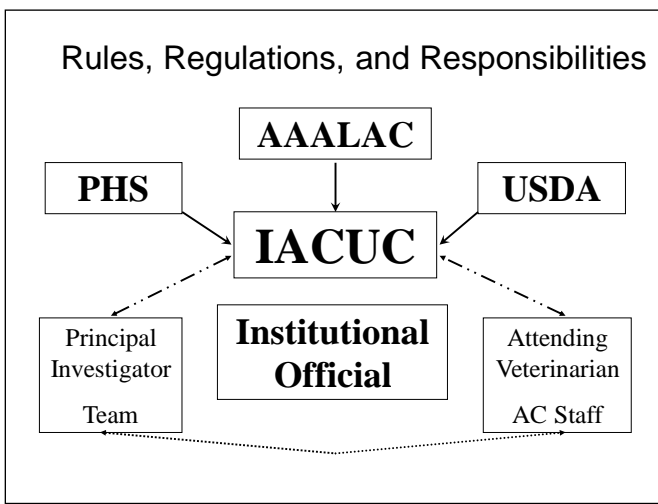
VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care,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

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

IX. 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.**

*For guidance throughout these Principles, the reader is referred to the [Guide for the Care and Use of Laboratory Animals](#) prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences.

**Published in the *Federal Register*, May 20, 1985, Vol. 50, No. 97, by the Office of Science and Technology Policy [FR Doc. 85-12059].



The Animal Welfare Act

- Public Law 89-544, 1966; as amended PL 91-579, 1970; PL 94-279, 1976; PL 99-198, 1985; PL101-624, 1990
- Animal Welfare Regulations, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Regulatory Enforcement and Animal Care (REAC), Code of Federal Regulations, Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare), Parts 1-4
- Annually, number of animals used according to categories of potential pain and distress:



Pain and/or Distress Classification System		
USDA Category	UofL Classification	Description
B	0	Obtained but not yet used
C	1	Procedures involving minimal (“needle-stick”) pain and distress
D	2	Procedures involving more than minimal but alleviated pain and/or distress
E	3	Procedures involving unalleviated* pain and/or distress

*Must include scientific justification for not alleviating potential pain and/or distress

Public Health Service Act

- Title 42, U.S. Code, Sections 289d, and Health Research Extension Act (PL 99-158, 1985)
- PHS *Policy on Humane Care and Use of Laboratory Animals* (intended to supplement IRAC Principles)
 - Agency for Healthcare Research and Quality
 - Centers for Disease Control and Prevention
 - Food and Drug Administration
 - Health Resources and Services Administration
 - Indian Health Service
 - National Institutes of Health
 - Substance Abuse and Mental Health Services Administration
- Office of Laboratory Animal Welfare (OLAW)
- Assurance of Compliance with the PHS Policy (“Assurance”) [UofL [A3586-01](#)]
- Refers to The Guide for the Care and Use of Laboratory Animals (the “Guide,” 2011) for specific guidance (see below).



Note: There are differences between these two sets of regulatory guidelines, but at UofL we abide by both because we must be registered with the USDA and we wish to continue to receive PHS funding.

AAALAC, International

- Formerly known as the Association for the Assessment and Accreditation of Laboratory Animal Care, International; changed name to simply the acronym in 2016
- Voluntary, non-profit, founded in 1965
- UofL is *Unit No. 000007*; **now the longest-running accredited institution in the world!**
- Uses the “Guide” as primary reference

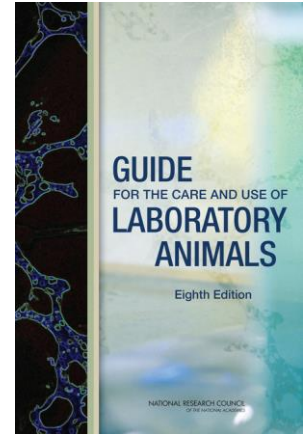


The Guide for the Care and Use of Laboratory Animals

- Institute of Laboratory Animal Resources (ILAR), Commission on Life Sciences, National Research Council (NRC). 2011. “The Guide”
- Embraces “*Performance Standards*” in five sections:
- *Key Concepts* (such as “should,” “must,” and “may”)
- *Animal Care and Use Program*
 - Regulations, Policies, and Principles
 - Program Management
 - Program Management Responsibility*
 - Personnel Management
 - Training and Education*
 - Occupational Health and Safety of Personnel*
 - Personnel Security
 - Investigating and Reporting Animal Welfare Concerns*
 - Program Oversight
 - The Role of the IACUC
 - Protocol Review*

*
**Pertinent
IACUC
Policy(ies)**
*

- Special Considerations for IACUC Review*
 - Post-Approval Monitoring*
- Disaster Planning and Emergency Preparedness
- *Environment, Housing, and Management* [Separate sections on *Terrestrial* and *Aquatic* Animals]
 - Environment
 - Microenvironment and Macroenvironment
 - Temperature and Humidity
 - Ventilation and Air Quality (or Water Quality)
 - Illumination
 - Noise and Vibration
 - Life Support System
 - Housing
 - Microenvironment (Primary Enclosure)
 - Environmental Enrichment
 - Sheltered or Outdoor Housing
 - Naturalistic Environments
 - Space
 - Management
 - Behavioral and Social Management*
 - Husbandry
 - Population Management*



- *Veterinary Care*
 - Animal Procurement and Transportation*
 - Preventive Medicine*
 - Clinical Care and Management*
 - Surgery*
 - Pain and Distress
 - Analgesia, and Anesthesia*
- *Physical Plant*
 - General Considerations*
 - Functional Areas
 - Construction Guidelines
 - Special Facilities
 - Surgery
 - Barrier Facilities
 - Imaging
 - Whole Body Irradiation
 - Hazardous Agent Containment
 - Behavioral Studies
 - Aquatic Species Housing
 - Security and Access Control*

Note: All animal acquisition must be through RRF, Penny White, 852-4605; See IACUC Policy, “Animal Acquisition”

- Euthanasia

Other Pertinent Laws

- Good Laboratory Practice Standards
 - Food and Drug Administration, Good Laboratory Practice Standards (21CFR58)
 - Environmental Protection Agency, Good Laboratory Practices for Pesticide Program (40CFR160) and for Toxic Substances (40CFR792)
- Endangered Species Act, 16 U.S.C. 1531
- Freedom of Information Act (FOIA), 5 U.S.C. 552
- Some State and Local Laws (few in Kentucky)

The Institutional Animal Care and Use Committee (IACUC)

IACUC Membership

- Researchers using animals
- Laboratory animal veterinarian
- Non-affiliated members (community representative)

IACUC Responsibilities

- Ensure humane treatment by reviewing proposals for use
- Semi-annual assessment of programs and facilities; report to Institutional Official (at UofL: Gregory Postel, M.D, Interim Executive Vice President for Health Affairs)
- Review all animal welfare concerns
- Suspend non-compliant activities
- Make recommendations to IO concerning issues associated with the Animal Care and Use Program

IACUC Proposal Review

- See IACUC Policies:
 - “Proposal Review and Approval”
 - “Modification of an Approved Proposal”
 - “Research Involving Collaborating Institutions”
 - “Proposal Expiration”
 - “Proposal Annual Renewal”
 - “Use of Tissue and Tissue Sharing”
 - “Oversight of Core Animal Laboratories”
 - “Use of Chicken/Avian Embryos”
 - “Research Studies Using Privately-Owned Animals”
- See Information Sheet, “Animal Number Justification”

- Integrated Research Information System (iRIS)

Designated Review System

- Pre-Review
 - Animal Care Specialist
 - Veterinarian
 - IBC/DEHS/Radiation – *as necessary*
- Reviewer Consultant(s)
- Designated Reviewer

Full Committee Review

Review Considerations

- Clearly justified need for conducting the study; “harm-benefit analysis”
- Clear, sequential description of procedures and observations to be performed for each experimental group
- Detailed description of all procedures, outlining appropriate methods of animal care and use
- Written consideration of all possible alternatives to potentially painful and/or distressful* procedures and why possible alternatives cannot be employed*
- Written assurance of lack of unnecessary duplication*
- Methods used to alleviate intended or unintended pain and/or distress, including criteria used for intervention (humane endpoints) regardless of scientific endpoints*

*specifically relate to the “three Rs”

¡Note!

- **All** animal use must be reviewed and approved by the IACUC BEFORE the work is actually conducted.
- **No** animal use (teaching or research) is authorized without PRIOR IACUC approval.
- **Annual Renewal** required (USDA)
- Proposal approval **Expires** in 3 Years – no exceptions, no mechanisms for “extensions”

Components of Barrier Practices

Traffic Limitations:

- Strict “no return” policy
- Limited personnel traffic

Personal Protective Equipment and Practices:

- Dedicated shoes or covers
- Gloves
- Sterilized clothing or outerwear
- Masks (surgical or N95)
- Hair bonnets

Facilities and Equipment:

- Positive ventilation
- Positively-pressurized IVCS
- Caging sterilized as "kits"
- HEPA-filtered change-out hoods
- Sterilization or decontamination of research equipment and supplies
- Designated "turnaround"/ isolation rooms

Facility Access Requirements

Requests for access authorization to RRF-controlled areas must be submitted to the Facilities Manager (Mr. Ralph Hornickel, 852-4604). Personnel must complete a designated form, which includes either their employee ID or “Cardinal Card” number. Before access is granted, the RRF verifies that the individual has:

- 1) been approved as a participant on at least one IACUC-approved *Proposal*,
- 2) attended the IACUC-mandated basic animal welfare training entitled, “Level II Training,” which includes access information and a discussion of security,
- 3) completed enrollment in the Occupational Health and Safety Program for animal handlers,
- 4) completed animal facilities-specific training.

Issues for Special Consideration

Alternatives

- *Proposals* must contain a written *narrative* describing the consideration of all possible alternatives to potentially painful and/or distressful procedures and the reasons why possible alternatives cannot be employed
- Remember: *Replacement* (Animal vs. Non-animal Models), *Reduction* (statistical rationale to animal number selection, assurance of lack of unnecessary duplication), and *Refinement* (skills and techniques, use of pain-relieving methods, other methods of reducing/eliminating animal pain/distress)
- Many resources/services available (see IACUC Information Sheet)

Pain and Distress

Definitions

- *Discomfort* – minimal change in homeostasis
- *Distress* – state requiring adaptation of homeostasis
- *Pain* – perception from nerve impulses by cerebral cortex of actual or potential tissue damage

Recognition and Alleviation: “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.” (IRAC Principle IV)

Use of Pain-Relieving Agents requires careful consideration of product selection as well as species-specific and animal-to-animal variability.

- Anesthetics – unconsciousness, analgesia, muscular relaxation
- Tranquilizers/Sedatives – reduce anxiety
- Analgesics – alleviate pain without loss of consciousness
- See IACUC Policy, “Use of Postoperative Analgesia”
- See Information Sheet, “Pain Scoring Using Response Variables”

Stages of Anesthesia

- I – analgesia, voluntary movement
- II – delirium, involuntary movement
- III – surgical anesthesia, unconsciousness
- IV – extreme CNS depression, resuscitation may be required

Assessment of anesthetic depth

- Pedal reflex, palpebral reflex, jaw tone, anal sphinctor tone
- Respiration -- ↑ depth or ↓ rate
- Heart rate

Use of DEA-Controlled Substances

- Registration (free for scientists)
- Record keeping requirements – careful records of inventory and use
- Storage and security to prevent theft

Neuromuscular blocking agents

- Must retain surgical anesthesia/unconsciousness
- Difficulty in monitoring since skeletal muscle reflexes are blocked

Survival Surgery

- Aseptic technique and Dedicated Facilities
- Major vs. Minor procedures
- Multiple Survival Surgery – must be justified scientifically
- Pre-, Intra-, and Post-operative practices
- IACUC Policy, “Performing Rodent Survival Surgery”
 - Dedicated Surgical Area
 - Aseptic Procedures
 - Animal Preparation
 - Surgeon Preparation
 - Sterile Instruments and Surgical Packs
 - “Tips Only” Technique
 - Maintaining the Surgical Field
 - Repetitive Surgical Procedures
 - Suture Materials/Methods
 - Anesthesia and Anesthetic Monitoring
 - Postoperative Treatment and Care
 - Perioperative Recordkeeping
 - Analgesia and Recognition of Pain
- See IACUC Policy, “Individual Animal Records”
- See Information Sheets:
 - “Perioperative and Anesthetic Records for Rodents”
 - “Monitoring the Effectiveness of Sanitation and Sterilization”

Euthanasia

American Veterinary Medical Association (AVMA) Guidelines on Animal Euthanasia (2013)

- Acceptable
- Acceptable with conditions – includes many commonly employed methods such as carbon dioxide and physical methods
- Unacceptable

Some Key Concepts:

- Adequate training – make sure you know what you are doing!
- Should not be performed in presence of other animals
- Technician considerations – ask for reassignment if euthanasia begins to weigh heavily
- **Methods to ensure death** – you **must** palpate for cessation of heartbeat; follow-up with a physical method (*e.g.*, cervical dislocation, bilateral thoracotomy, vital organ removal)
- See Information Sheets
 - “Use of Carbon Dioxide for Rodent Euthanasia”
 - “Preparation and Use of MS-222”
 - “Euthanasia of Zebrafish”

Humane Endpoints

- See IACUC Policy, “Humane Endpoints”
- Specific criteria for intervention (including euthanasia) should be outlined in *Proposal*
- Unexpected pain/distress should be considered
- “Death-as-an-endpoint” is rarely acceptable
- “Will consult with veterinarians” also rarely acceptable – if an animal is observed at midnight to require intervention, waiting until a veterinarian can assess is not humane or practical; thus the need to have detailed, objective criteria for intervention (usually euthanasia)

Social Housing

- IACUC Policy, “Social Housing of Animals”
- Most laboratory animals are social in nature and therefore must be housed in pair or groups unless there is a compelling reason for isolation:
 - Research objectives
 - Scientific necessity, as described in Proposal (*e.g.*, monitoring fluid intake)
 - Attrition of an established research group
 - Acquisition of a single animal or birth of a single gender in a litter
 - Veterinary care
 - Individual animal treatment
 - Immediate post-surgical period (up to 14 days or suture removal)
 - Behavioral incompatibility
 - Fighting
 - Breeding male
 - Peri-parturient female; considered “social” once litter is born

Laboratory Use and Satellite Housing

- See IACUC Policies:
 - “Transporting Animals to Research Laboratories”
 - “Animal Security in Investigator Laboratories”
 - “Laboratory and Satellite Rodent Housing”
 - “PI-Maintained Non-Mammalian Housing”
- See Information Sheets:
 - “PI-Maintained Aquatic Laboratories”
 - “Monitoring the Effectiveness of Sanitation and Sterilization”
- All require description and justification within the “Special Housing” section *each pertinent Proposal*
- Animals should be retained in RRF-provided home cage unless special enclosures are approved

Terminology	Timeframe	Record Requirements
Day Use	< 12 hrs	No special requirements
Overnight	12-24 hrs	Emergency contact numbers
Temporary Satellite Housing	24-72 hrs	+ Light cycle control, dates noted on cage cards, daily documentation of census, max/min room temperature, food/water/health checks
Satellite Housing	> 72 hrs	+ daily max/min relative humidity and an approved Satellite Housing Area Description (SHAD)

Occupational Health and Safety Program

- Use of hazardous species
 - Non-Human Primate tissues (treat as if infected with Blood-borne Pathogens)
 - Carnivores (Rabies vaccination available)
 - Sheep (Q-fever policy for pregnant ewes)
- Use of hazardous agents
 - Biological Hazards – Biological Safety Officer (BSO, Dr. Torsten Hopp) and the Institutional Biosafety Committee (IBC)
 - Chemical Hazards – Industrial Hygiene Manager (Dr. Torsten Hopp)
 - Radiation Hazards – Radiation Safety Officer (RSO, Sarah Hughes) and the Radiation Safety Committee (RSC)
 - **Special Animal Safety Protocols (SASPs) [See Information Sheet]**
 - PI Responsible for posting SASP on Animal Holding Room door
 - Special instruction card on affected cages
 - red = biological hazard
 - orange = chemical hazard
 - **Unacceptable:** SASP on door with no red/orange card; red/orange card on cage(s) with no SASP on door
- Two-Part Enrollment
 - See IACUC Policy, “Individual Risk Assessment and Medical Surveillance”

1. Training

- Read “Occupational Hazards Associated with the Care and Use of Laboratory Animals”

- Take the associated Quiz
- Repeat every 3 years

2. *Individual Risk Assessment*

- Complete “Periodic Animal Contact Health Survey”
- Repeat if health conditions change (including pregnancy)
- Repeat at least every 3 years

Reports of Concern

- “Whistle-blowers,” but also conditions that can be improved that are in compliance
- IACUC Policy: “Responding to Reported Deficiencies in Animal Care and Treatment”
- Guaranteed confidentiality and freedom from repercussion
- University Compliance Helpline: 877-852-1167; <http://louisville.edu/compliance/helpline>

Take-Home Message #1: Animal research models must be as free from pain and distress as possible to yield robust research data. Humane animal care is a scientific imperative.

Take Home Message #2: Always remember the Three R’s.

Take Home Message #3: If you don’t KNOW, then ASK.