

# 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?

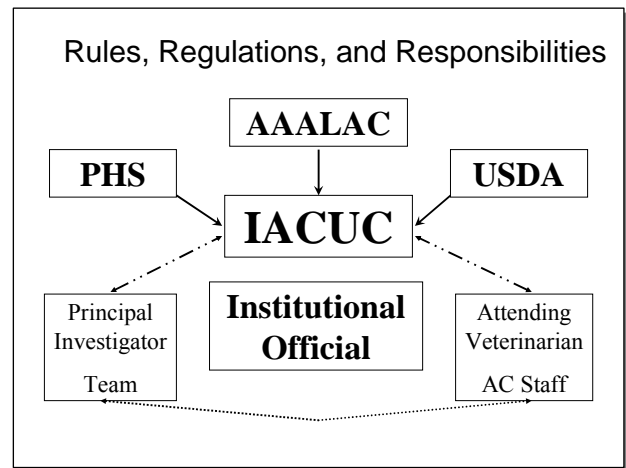
- Animals continue to play a prominent role in scientific investigations.
- The use of animals is controversial.
- The public’s perception of the use of research animals is often skewed.
- The use of animals has therefore become highly regulated.

## Regulatory Overview

### “The Three R’s”

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

- **Replacement** – ethical obligation to use non-live-animal models whenever possible (*section titles: comparative substitution, modes of absolute and relative replacement, principles of replacement, use of tissue culture, use of micro-organisms*)
- **Reduction** – ethical obligation to use only the number of animals necessary and avoid unnecessary duplication (*section titles: 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*)
- **Refinement** – ethical obligation to improve the acquisition, housing, care, and procedure used with animals to reduce any potential pain and/or distress (*section titles: 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*)



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

### **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 (see box at right).

**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)
- Office of Laboratory Animal Welfare (OLAW)
- Assurance of Compliance with the PHS Policy (“Assurance”)
- Refers to *The Guide for the Care and Use of Laboratory Animals* (“The Guide,” 1996) for specific guidance (see below).

USDA Annual Report and IACUC Classification of Potential Pain and Distress		
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 distress
E	3	Procedures involving unalleviated* pain and distress
*Descriptions must include scientific justification for not alleviating potential pain and distress		

**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**

- Association for the Assessment and Accreditation of Laboratory Animal Care, International
- Voluntary, non-profit, founded in 1965
- Uses the “Guide” as primary reference

**“The Guide”**

- Institute of Laboratory Animal Resources (ILAR), Commission on Life Sciences, National Research Council (NRC). 1996. *Guide for the Care and Use of Laboratory Animals*.
- Embraces “performance standards” in four sections:
- Institutional Policies and Responsibilities*
  - Monitoring the Care and Use of Animals
  - Veterinary Care
  - Personnel Qualifications and Training
  - Occupational Health and Safety of Personnel
- Animal Environment, Housing, and Management*
  - Physical Environment
  - Behavioral Management
  - Husbandry
  - Population Management
- Veterinary Medical Care*
  - Animal Procurement and Transportation
  - Preventive Medicine
  - Surgery
  - Pain, Analgesia, and Anesthesia
  - Euthanasia
- Physical Plant*
  - Functional Areas
  - Construction Guidelines
  - Facilities for Aseptic Surgery

**Note:** IACUC Policy on Housing in Laboratories

**Note:** All animal acquisition must be through RRF, Mike Marsee, 852-4605

**Note:** IACUC Policy on Animal Transportation

## 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 Responsibilities*

- Ensure humane treatment by reviewing proposals for use
- Semi-annual assessment of programs and facilities; report to Institutional Official (at UofL: Larry Cook, MD, 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 Membership*

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

**Notes:** 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”

### *IACUC Proposal Review*

- Submission to IACUC Coordinator
- “Pre-veterinary Review”
- Secondary Reviewers
- Primary Reviewer
- Chair Approval
- Full Committee Review

### *Commonly-Identified Deficiencies during IACUC Semi-Annual Laboratory and Facility Inspections:*

#### Monitoring Animal Care and Use:

- Laboratory site inconsistent with *Proposal*
- Incomplete Level II Training and/or OHSP enrollment
- Project participants not IACUC-approved (included a *Proposal*)
- Improper storage or recordkeeping for controlled substances
- Animal number updates needed for breeding colonies

#### Anesthesia and Analgesia:

- Use of chemical-grade anesthetic agents
- Questionable or undocumented use of post-operative analgesics
- Use of appropriate but not IACUC-approved anesthetic regimens

#### Occupational Health and Safety of Personnel:

- Incomplete or unnecessary “red” cards
- Use of expired Special Animal Safety Protocol (SASP)
- Unsecured gas cylinders
- Uncertified biosafety cabinets / fume hoods
- Use of hazardous agent not described in IACUC-approved *Proposal*

#### Surgery:

- Incomplete surgical records
- Surgical instruments not appropriately sterilized

#### Husbandry:

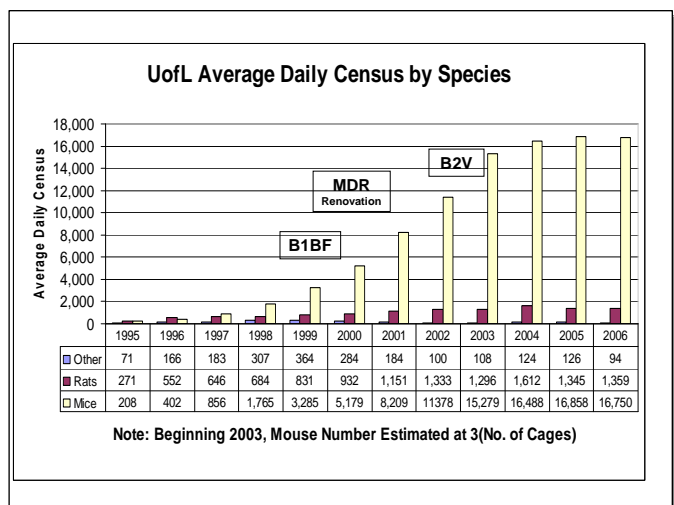
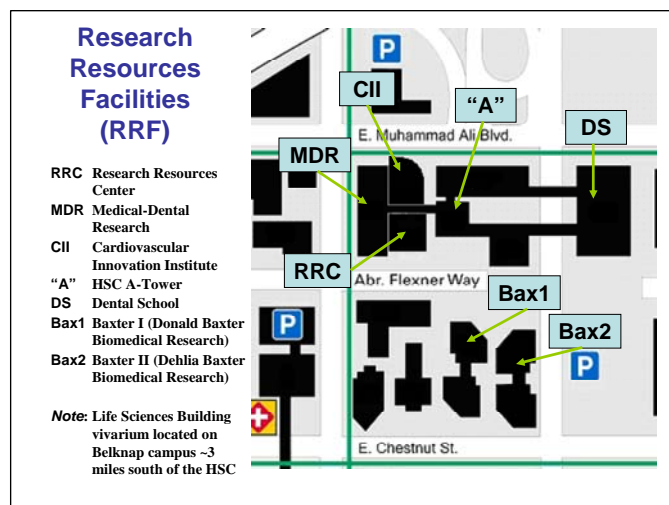
- Extraneous research equipment in animal housing room; supplies not kept in an appropriate bin

- Unsanitizable equipment or furniture in animal or procedure rooms
- Lack of appropriate (RRF-produced) cage cards or cards identifying an expired *Proposal*

## The UofL Animal Care and Use Program

### Compliance Information

<b>USDA</b>	Registration number	61-R-001-01
<b>PHS/OLAW</b>	Assurance number	A3586-01
	Assurance period	31 August 2005 – 31 August 2009
<b>AAALAC</b>	Unit number	000007
	Most recent Site Visit	19-20 March 2007
	Most recent notification letter	12 July 2007
	Accreditation Status	Full



### 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

- 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)

- Resources/Services for Consideration of Alternatives:
  - National Agricultural Library
  - Johns Hopkins Center for Alternatives to Animal Testing
  - Rockefeller University Laboratory of *In Vitro* Toxicological Assay Development
  - Fund for the Replacement of Animals in Medical Experiments
  - Animal Welfare Information Center (AWIC)
  - Literature databases (Medline, *etc.*)
  - IACUC/RRF

## 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

### Survival Surgery

- Aseptic technique and Dedicated Facilities
- Major vs. Minor procedures
- Multiple Survival Surgery – must be justified scientifically
- Pre-, Intra-, and Post-operative practices

### Euthanasia

*American Veterinary Medical Association (AVMA) Guidelines on Euthanasia*

- Acceptable
- Conditionally acceptable (*e.g.*, physical methods)
- Unacceptable

### Stages of Anesthesia

- I – analgesia, voluntary movement
- II – delirium, involuntary movement
- III – surgical anesthesia, unconsciousness
- IV – extreme CNS depression, resuscitation 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
- Record keeping requirements
- Storage

### Neuromuscular blocking agents

- Must retain surgical anesthesia/unconsciousness
- Difficulty in monitoring

**Note:** IACUC Policy on Post-Operative Analgesia

**Note:** IACUC Policy on Rodent Survival Surgery

**Note:** IACUC Policy on Expired Materials and Pharmaceutical-Grade Medications

### Key Concepts:

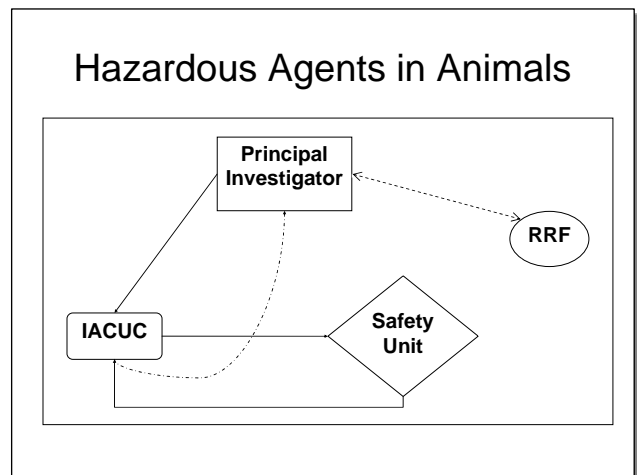
- Adequate training – make sure you know what you are doing!
- Should not be performed in presence of other animals
- Technician considerations – ask for reassignment if euthanasia begins to weigh heavily.
- Methods to ensure death – you must palpate for cessation of heartbeat; follow-up with a physical method (e.g., cervical dislocation, bilateral thoracotomy, vital organ removal)

### 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

### Occupational Health and Safety Program

- Two-Part Enrollment
  - **Training:** Read “Occupational Hazards Associated with the Care and Use of Laboratory Animals” and take the associated Quiz (re-read at least every 3 years)
  - **Individual Risk Assessment:** Complete “Periodic Animal Contact Health Survey” every 3 years
- Special considerations for use of hazardous agents
  - Biological Hazards – Biological Safety Officer (BSO, Dr. Carol Whetstone) and the Institutional Biosafety Committee (IBC)
  - Chemical Hazards – Laboratory Safety Coordinator (LSC, Erin Foley)
  - Radiation Hazards – Radiation Safety Officer (RSO, Ken Helm) and the Radiation Safety Committee (RSC)
  - Special Animal Safety Protocols (SASPs)
- Special consideration for using hazardous species
  - Non-Human Primate tissues (treat as if infected with Blood-borne Pathogens)
  - Carnivores (Rabies vaccination available)
  - Sheep (Q-fever policy)



### Reports of Concern

- “Whistle-blowers”
- IACUC Policy: “Responding to Reported Deficiencies in Animal Care and Treatment”
- Guaranteed confidentiality and freedom from repercussion

**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.