

# Using Animal Subjects in Research



Office for the Protection of Research Subjects (OPRS)

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**About the Source Material**

The Collaborative Institutional Training Initiative (CITI) web based education program, developed by the University of Miami and the Fred Hutchinson Cancer Research Center, offers training in Human Subjects Research, the Responsible Conduct of Research, and Good Clinical Practice. CITI is currently used by over 1130 participating institutions and facilities from around the world and offers online course material in more than seven different languages. CITI RCR was developed with public funds and thus allowed access to material used to create these booklets.



## Introduction to Using Animal Subjects in Research

Animals are used in research to improve knowledge in the biomedical sciences to benefit human health and animal health. Opponents of animal research maintain that animals should not be utilized in research designed to benefit humans. Opposition to the use of animals as a precursor to human research has resulted in a dramatic increase in alternative technology, thus greatly reducing the use of animals in research.

Research with animals is strictly regulated at the institutional and federal level. Investigators are encouraged to explore alternatives before using animal subjects. Proposed research using animals must have research value and must justify the use of animals, rather than other means to answer the question. Animal studies must be conducted for a societal goal and in accordance with federal and institutional welfare policies.

The objective of this booklet is to provide information about the ethical and regulatory requirements associated with the use of animal subjects in research. After reading this booklet, readers will have a better understanding of the responsibilities involved when research is conducted on laboratory animals. Case studies and reference lists are also provided.

# The 3Rs of Research with Lab Animals

Investigators who choose to conduct research with animals should carefully design the study to minimize or eliminate the use of whole animals. If animals are utilized, investigators should minimize invasive procedures whenever possible. The “**3Rs**”; **Reduction, Replacement and Refinement**<sup>1</sup> approach, described below, consist of three strategies to reduce discomfort or eliminate animal use entirely.

## ▪ **Replacement**

The concept of “replacement” refers to using alternatives to animal subjects when designing research protocols. For example, tissue culture systems or computer simulations may offer alternatives. Replacement only works when it provides scientifically valid results and is appropriate for the question being studied.

## ▪ **Reduction**

The concept of "reduction" refers to the strategies used to reduce the number of animals used in research. For example, statistical analysis can be used to determine an adequate sample size for a research protocol. It is important to reduce the unnecessary use of animals beyond what is required for a valid outcome while preventing a situation where too few animals are used to adequately test a study hypothesis.

## ▪ **Refinement**

The concept of “refinement” refers to continually seeking ways to modify experimental methods in order to minimize the number of animals used in a study and resort to the most humane approach possible. Refinement techniques include:

- Modifications to procedures to reduce stress or eliminate pain
- Application of a strategy that permits the experimental animal to act as its own control, increasing the reliability of the measurements and decreasing the need for an independent control group
- Periodic evaluation of data during the collection process to determine whether the sample size can be reduced or study question has been answered

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<sup>1</sup> “*The Principles of Humane Experimental Technique*” (1959) William Russell and Rex Birch

- Environmental enrichment (make the habitat more interesting or stimulating for animals)
- Application of noninvasive sensing technologies

## Before Conducting Animal Research

A good research design includes both ethical and scientific considerations. When designing a study that will utilize animal subjects, the following steps should be taken to ensure research is ethically and scientifically sound:

- Consult with peers who conduct research with animals
- Conduct a literature search for rationale for use of animal subjects and to avoid duplicating work in print
- Evaluate animal research facilities at your institution or lab
- Familiarize yourself with animal welfare policies, procedures, and regulations at your institution
- Carefully select the appropriate species and number of animals to be used in your study
- Statistically establish objective endpoints, number of experiments to be performed, and adequate numbers of controls versus experimental subjects

## Submitting a Research Protocol to IACUC

Faculty, staff, and students must adhere to all federal policies related to animal welfare. All research involving the use of vertebrate animal subjects must be approved by an Institutional Animal Care and Use Committee (IACUC). At USC, a Protocol Synopsis Form (<http://www.usc.edu/hsc/dar/iacuc/forms>) must be completed and signed by the Principal Investigator and submitted to the IACUC coordinator.



The PI is required to describe the following in the Protocol Synopsis:

- Rationale and purpose for the use of particular animals
- The names of all individuals who will handle animals
- Justification for the species and the number of animals requested (whenever possible, the number should be justified statistically)
- Any potentially painful/distressful procedures that will be performed on the animals

- Availability or appropriateness of alternatives (less-invasive procedures, use of other species with less ethical cost, isolated organ preparation, cell or tissue culture, or computer simulation)

## **Making Changes to the Protocol**

Changes to the procedures used in already approved animal research protocol must be reviewed and then approved by the IACUC prior to implementation. Resubmitting the protocol to the IACUC ensures that the study conforms to the most up-to-date policies on animal welfare in a research setting. Performing unapproved procedures is expressly prohibited and will be considered non-compliant according to IACUC and federal regulations. The conduct of unapproved procedures will usually result in suspension of the protocol.

## **Training Research Personnel**

The USDA [Animal Welfare Act](#) and HHS [Public Health Service Policy](#) require that all researchers conducting animal research, complete a basic training session regarding humane care and use of animals in a research environment. USC's IACUC website (<http://www.usc.edu/hsc/dar/iacuc/>) offers researchers a complete series of web courses, audiovisual presentations, and self-exams based on the species listed and procedures described in the approved Protocol Synopsis.

## **Care and Handling of Animal Subjects**

Proper management of animal housing is essential to animal well-being and quality of research data. A good animal management program provides housing and care that allow animals to maintain good health and minimizes variations in the study that can affect research results.

Many factors should be considered in planning adequate and appropriate physical and social environment, housing, space, and management for animal subjects. These include:

- The species, strain, and breed of the animal and individual characteristics, such as sex, age, size, behavior, experiences, and health
- The ability of the animals to form social groups with conspecifics through sight, smells, and possibly contact, whether the animals are maintained singly or in groups
- The design, construction, and maintenance of appropriate housing
- The availability or suitability of enrichments

- Species-appropriate, dietary requirements
- Appropriate and approved use of restraints
- The maximum allowable number of animals per cage

## **Avoiding Animal Subject Discomforts**

The IACUC has implicit authority and responsibility to carefully review proposed protocols for scientific rationale and merit. The Animal Care and Use Committee prohibits certain procedures with laboratory animals unless compelling justification is provided by the investigator

Analgesia (pain relievers) should be used when research procedures cause more than momentary pain and distress to the subjects. The analgesia strategy must be species-appropriate, approved by the IACUC, and conform to veterinary best practices.

Studies in which the use of analgesics interferes with a study endpoint must receive exception from the IACUC.

## **Euthanasia**

Euthanasia of animals must occur with minimal pain and distress, and the exact method of euthanasia must be described in the protocol and be approved by the IACUC. There are recommended practices available in the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia (USC's Investigator Manual from the Department of Animal Resources and Institutional Animal Care and Use Committee can be found here: [http://www.usc.edu/hsc/dar/private/documents/Investigator\\_Manual.doc](http://www.usc.edu/hsc/dar/private/documents/Investigator_Manual.doc)). The proper training of personnel performing the euthanasia is critical.

## **Occupational Health and Safety**

Animal contact is accompanied by certain disease risks and the possibility of developing allergies. Certain diseases are not only transmissible from primates to humans (zoonotic transfer), but also from humans to non-human primates. It is very important that any human-animal interaction at clinical laboratories be properly planned and performed as per regulations, and approved by the IACUC and clinical professionals managing the area.

## **Reporting Misuse, Mistreatment or Non-Compliance**

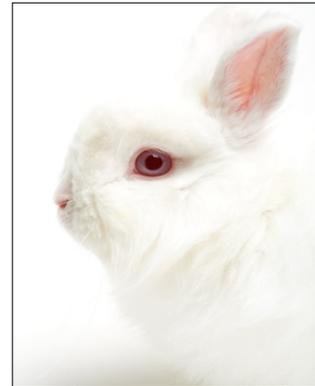
If one observes misuse or mistreatment of animals, or witnesses procedures that do not comply with federal regulations or guidelines, the incident should be immediately reported to a member of the IACUC, veterinarian of the institution, or the Office of Compliance hotline.

Once an allegation of mistreatment, misuse, or noncompliance is received, the IACUC will perform a review and if warranted, investigate the allegations. The IACUC has the regulatory authority to suspend a study to protect animals or people.

## Conclusion

New technologies and animal models of disease have led to major reductions in the number of animals needed for research. As the research community strives to develop alternatives to the use of animal subjects, society will continue to benefit from the discoveries that are currently only made possible through the use of animal subjects.

Research on animals is a privilege requiring integrity and the utmost professionalism. Without sound research practices, both animals and humans are at risk, valuable time and resources are wasted, careers are jeopardized and the acceptance of valid scientific research results are delayed or invalid.



# Case Studies

## I. Ethics in Animal Care and Use

Adapted from "Getting Results" by M.J. Beabeau and K.D. Pimple

Jenny Ito is a second year graduate student working in the biology lab of Chris Holzer. Ito has been overseeing an experiment that Holzer designed to determine whether a special anti-bacterial coating can reduce the incidence of infection associated with surgical pins. With Holzer's help, Ito has inserted pins into the right leg of thirty rabbits; fifteen of the pins are standard surgical pins, and fifteen have the anti-bacterial coating. About one-quarter inch of each pin protrudes the skin. Ito also inoculated all of the rabbits at the insertion point with *staphylococcus aureus* bacteria and routinely administers morphine at 5 mg/kg to alleviate any discomfort the rabbits may be experiencing because of the procedure. For almost a month, Ito has cared for the rabbits and recorded her observations, watching for any sign of distress or infection.

In her weekly meeting with Holzer, Ito reports that none of the rabbits seem to be particularly uncomfortable, and none of them show any signs of infection.

Holzer seems impatient. "If we don't get an infection, we won't learn anything. It would be a shame to have put these rabbits through this, not to mention wasting all your time, without getting some results, I want you to help things along a bit. I want you to inoculate all of the rabbits with *pseudomonas aeruginosa*. We'll see what happens then". Ito hesitates. "The protocol specifies *Staphylococcus*, Dr. Holzer".

Holzer brushes this off. "It's only a small change. We've been approved to run the risk of infecting these rabbits; all we're going to do is give the process a little boost."

1. What regulations or ethical guidelines does Dr. Holzer's suggestion violate?
2. Who should Jenny contact to voice her concern?
3. Should Jenny just do as Holzer says?
4. What would you do?

## II. Safety and Effectiveness of a Biomedical Implant

By Elizabeth Myers and Stephanie J. Bird

A design engineer, Louise Chandler has developed a new design for a hip prosthesis. The new design represents a major departure from previous designs. The shape and the materials are innovative and have not been used before. Dr. Chandler wants to test the implant in an animal before it is used in humans. After reviewing the literature, she finds that dogs have been used previously to test joint replacements. For most designs, results in dogs were subsequently shown to be very similar to results in humans.

However, she finds one case that is troubling; several years ago, another innovative design was tested in dogs and it performed very well in studies with dogs. Unfortunately, the prostheses had a very high failure rate in humans.



Dr. Chandler also finds many reports in the literature of machines that simulate joints to test new prosthesis designs. She does not have these sophisticated pieces of equipment available to her.

1. Should Dr. Chandler test her new design in dogs, despite the one troubling study from the literature?
2. What considerations do you think Dr. Chandler should use to aid her in this decision?
3. What alternatives might Dr. Chandler have?
4. How could she make her study more ethically and scientifically sound?
5. Would you want to see her new design used in humans?
6. What else should precede human testing?

# Resources

**USC Office for the Protection of Research**

**Subjects:**

<https://oprs.usc.edu/>

**USC Office of Compliance Hotline:**

<ooc.usc.edu/Hotline.cfm>

**USC Department of Animal Resources:**

[www.usc.edu/hsc/dar/](http://www.usc.edu/hsc/dar/)

**Use of Animals in Research:**

[ethics.iit.edu/emerging/bme/animal.html](http://ethics.iit.edu/emerging/bme/animal.html)

**Scientists Center For Animal Welfare:**

[www.scaw.com/](http://www.scaw.com/)

**Alternatives to Animal Testing:**

[toxnet.nlm.nih.gov/altbib.html](http://toxnet.nlm.nih.gov/altbib.html)

**Model Organisms for Biomedical Research:**

[www.nih.gov/science/models/](http://www.nih.gov/science/models/)

**Virtual Anesthesia Machine:**

[vam.anest.ufl.edu/](http://vam.anest.ufl.edu/)

**CITI Program:**

[www.citiprogram.org](http://www.citiprogram.org)

**John Hopkins Center for Alternatives to Animal Testing:**

<http://caat.jhsph.edu/>

# USC Contacts

## Office for the Protection of Research Subjects

3720 South Flower Street, Third Floor  
Los Angeles, CA 90089-0706  
Tel (213) 821-1154  
Fax (213) 740-9299  
E-mail: [oprs@usc.edu](mailto:oprs@usc.edu)  
<https://oprs.usc.edu/>

## Health Sciences Institutional Review Board

General Hospital, Suite 4700  
1200 North State Street  
Los Angeles, CA 90033  
Tel (323) 223-2340  
Fax (323) 224-8389  
E-mail: [irb@usc.edu](mailto:irb@usc.edu)  
<https://oprs.usc.edu/hsirb/>

## University Park Institutional Review Board

Credit Union Building (CUB), Suite 301  
3720 S. Flower Street  
Los Angeles, CA 90089  
Tel (213) 821-5272  
Fax (213) 821-5276  
E-mail: [upirb@usc.edu](mailto:upirb@usc.edu)  
<https://oprs.usc.edu/upirb/>

## Office of Research

Credit Union Building, Suite 325  
3720 S. Flower Street  
University of Southern California  
Los Angeles CA 90089-4019  
Tel (213) 740-6709  
Fax (213) 740-8919  
E-mail: [vice.president.research@usc.edu](mailto:vice.president.research@usc.edu)  
<http://www.usc.edu/research/>

## CITI Helpdesk

Tel (213) 821-5272  
E-mail: [citi@usc.edu](mailto:citi@usc.edu)  
[www.usc.edu/admin/provost/oprs/citi](http://www.usc.edu/admin/provost/oprs/citi)

## iStar Technical Help

Tel (323) 276-2238  
E-mail: [istar@usc.edu](mailto:istar@usc.edu)  
Web: <http://istar-chla.usc.edu>

## Office of Compliance

3500 Figueroa Street  
University Gardens Building, Room 105  
Los Angeles, CA 90089-8007  
Tel: (323) 740-8258  
Fax: (213) 740-9657  
E-mail: [complan@usc.edu](mailto:complan@usc.edu)  
<http://www.usc.edu/admin/compliance/>

## USC Stevens Institute for Innovation

3740 McClintock Ave. Hughes EEB 131  
Los Angeles CA 90089  
Tel: (213) 821-5000  
Fax: (213) 821-5001  
<http://stevens.usc.edu/>

## Health Research Association (HRA)

1640 Marengo Street, 7th Floor  
Los Angeles, CA 90033  
Tel (323) 223-4091  
Fax (323) 342-0947  
Web: <http://www.health-research.org/>

## IRB Student Mentor

Tel (213) 821-1154  
E-mail: [irbgara@usc.edu](mailto:irbgara@usc.edu)  
<https://oprs.usc.edu/education/mentor/>

## Office of Contracts and Grants – UP

Credit Union Building (CUB), Suite 303  
3720 S. Flower Street  
Los Angeles, CA 90089  
Tel: (213) 740-7762  
Fax: (213) 720-6070  
<http://www.usc.edu/research/dcg/>

## Office of Contracts and Grants – HSC

1540 Alcazar Street, CHP 100  
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