

## Appendix 1. IACUC Animal Certification Test

Welcome to the "Working with the IACUC" course for investigators, staff and students performing research or faculty and other personnel performing teaching that involves the use of animals.

*Successful completion of this certification is measured by receiving at least an 85% on the associated Blackboard quiz. You must retake the quiz until you achieve this minimal score.*

### Course Objectives

After completing this course, the user should be able to

- 1 Name the federal agencies that regulate the use of animals in research, teaching, and testing.
- 2 Name the *Guides* developed to help scientists and IACUCs provide humane care and use of animals.
- 3 Summarize the scientists' responsibilities when using animals in research, teaching, and testing
- 4 Incorporate reduction, replacement, and refinement (the 3Rs) into your research, teaching, and testing activities
- 5 Effectively
  - assure that personnel are trained and qualified
  - recognize and alleviate pain and distress
  - select and use properly anesthesia, analgesia and sedation
  - use properly aseptic technique in surgery

By **law**, an institutional committee must review all aspects of the animal care and use program. This committee is most commonly referred to as the "**Institutional Animal Care and Use Committee**" or IACUC. Your institution has its own IACUC, and Dr. Harper is the chair of this committee.

The IACUC is responsible for making sure that all federal laws, regulations and policies are followed when investigators perform animal research. The IACUC has many jobs including

**Reviewing** and approving animal use protocols submitted by investigators.

**Monitoring** the animal care and use program by conducting thorough reviews of the program and inspections of the animal facilities semiannually.

Federal regulators regard the IACUC as an essential partner in ensuring compliance with animal welfare laws and guidelines. In effect, the IACUC functions as the self-regulating body for animal research on behalf of the institution. Because the IACUC is such a critically important component of the animal care and use program, evaluating the effectiveness of the IACUC often serves as a barometer of the quality of the entire animal care and use program.

An effective IACUC protects both the individual investigator and the institution, while inspiring confidence in the general public that animal research is being performed in an ethical manner. Research utilizing animals is a privilege, not a right, and the IACUC must do its part to make sure that animal research is performed according to the highest standards. By assuring that animal research complies with animal welfare laws and guidelines, the IACUC ensures that animals are not subjected to unnecessary pain and distress and protects both the investigator and the institution.

The entire system is built on trust. However, a single incident of serious noncompliance with animal welfare regulation or guidelines can jeopardize the entire institution's privilege of conducting animal research.

*Answer questions 1-2 on the quiz.*

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**To what animals do the USDA Animal Welfare Act Regulations apply?** Here is the definition of an animal given in the regulations:

"Animal 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 not used for research purposes and other farm animals, such as, but not limited to livestock or poultry used or intended for use 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."

The second important agency involved in regulating animal use is the Department of Health and Human Services, which is the home of the **Public Health Service (PHS)**. Passed by Congress in 1985, the **Health Research Extension Act** directed PHS to provide guidelines for animal research. The **Office of Laboratory Animal Welfare (OLAW)** is responsible for monitoring institutional compliance with PHS policy and guidelines. OLAW relies primarily on two documents for judging compliance, both of which are very important to animal research. The first, fairly brief one, is the **PHS Policy on Humane Care and Use of Laboratory Animals**. It incorporates nine **U.S. Government Principles For The Utilization And Care Of Vertebrate Animals Used In Testing, Research, and Training** that must be considered when institutions receive support from U.S. Government agencies. The second, lengthier document is the **Guide for the Care and Use of Laboratory Animals** (usually called the *Guide*). These two documents together provide important information sometimes collectively called "PHS Policy." Compliance with PHS Policy is a required condition for receiving PHS support for activities involving vertebrate animals.

*Answer questions 3-4 on the quiz.*

Consulting with the veterinarian is an important part of filling out the animal forms. The USDA Animal Welfare Act Regulations stipulate that if procedures on animals are proposed that may cause more than momentary or slight pain or distress to animals, consultation with the attending veterinarian or his or her designee (a veterinarian with training or experience in laboratory animal medicine) must occur in the planning of those procedures. This is a good time to get advice on the latest medications and anesthetics, as well as tips on new procedures and ways to reduce pain or suffering.

**"How will the proposed use of animals contribute to human or animal health, the advancement of knowledge, or the good of society?"** This question or a variant appears in almost all animal protocol forms. This is a very important issue because you are asking for the privilege of using animals for procedures that rarely will benefit them individually and might result in their deaths. In general, there must be a compelling potential for benefit to warrant the use of animals. Another important question on protocol forms is **"What is the experimental design of the animal studies planned?"** In responding, keep in mind that the IACUC needs to understand the proposed use of animals.

The next module will discuss the importance of demonstrating that it is really necessary to use animals in the project--that use of other "non-animal" models will not suffice. Having shown that you must use animals, you will have to justify your choice of species. Federal regulations and the *Guides* specify that species should be chosen because of their "appropriateness." So, for example the *PHS Policy* states that **"The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results."** In other words, the species is the best one to accomplish the aims of the project.

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### Reasons why a given species is particularly appropriate may include

- ✓ **The presence of previous work in the literature that validates the use of a particular species in an animal model of a human or animal disease.**
- ✓ **The existence of a large body of previous laboratory data that would have to be repeated if another species was used instead.**
- ✓ **Characteristics of the species that render it uniquely suited to the proposed research.**
- ✓ **Size, availability and cost.**
- ✓ **Availability of reagents or research tools unique to that species.**

You should always use the most appropriate species in a research project, but, when two or more species are equally appropriate, IACUCs may ask that you use the least sentient ("aware") species capable of providing the needed data. Although there is no scientific consensus regarding the hierarchy of sentient species, many people believe that animals should be utilized starting at the bottom and moving toward the top of the following list:

- Apes (orangutans, gorillas)
- Monkeys (baboons, rhesus monkeys, marmosets, tamarins)
- Larger animals commonly kept as pets such as dogs and cats
- Larger animals such as pigs and goats commonly used as farm animals
- Rabbits
- Rodents (guinea pigs, hamsters, rats, mice)
- Non-mammalian vertebrates (poultry, amphibians, reptiles, fish)
- Invertebrates (crustaceans, slugs, insects, arachnids, worms, etc.)
- Single cell organisms (yeast, bacteria)

There may be other good reasons for avoiding the use of particular species even though it is otherwise appropriate. For example, many primates are endangered. Their use in research reduces the already small numbers left in the world. One can easily argue this is a good reason to avoid using them.

You will also be asked to request a certain number of animals, and justify why you need that number. The IACUC realizes that it can be difficult to provide such information in advance, but the law requires them to review the number of animals to be used.

### *Answer questions 5-7 on the quiz.*

Now is a good time to address the very important concept of alternatives. We've already touched upon it during the discussion of the importance of using the most appropriate animal species possible and during our discussion of justifying animal numbers requested.

In 1959, two British scientists, William Russell and Rex Burch, urged all animal researchers to follow a policy of minimizing animal pain and use. They described three important concepts now known widely as the "three Rs." These concepts are **replacement, reduction and refinement**. The purpose of these concepts is to minimize animal use and pain or distress while still achieving the critical scientific objectives that lead to advances in health and medicine.

The first "R" is **replacement**. Replacement is simply replacing the use of animals with non-animal techniques. Examples of non-animal techniques that can sometimes replace animals include

- ✓ **Computer models.**
- ✓ **Cell culture or tissue culture systems.**
- ✓ **In vitro assays.**

Unfortunately, it is not very common for any of the above alternate systems to adequately replace animals in experiments. However, it does happen, and consideration should always be given to non-animal systems.

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The second "R" is **reduction**. Reduction is simply reducing the number of animals used. Examples of reduction include

- ✓ **Limiting group sizes** to the minimum needed to obtain statistically significant data.
- ✓ **Performing multiple experiments simultaneously** so that the same control group can be used for all the experiments.
- ✓ **Sharing tissues** with other investigators so that additional animals are not needed.
- ✓ **Designing experiments so that animals serve as their own controls.**
- ✓ **Using newer instrumentation** that improves precision and reduces the number of animals needed per data point.

The last "R" is **refinement**. As opposed to reducing the number of animals used, refinement refers to changing experiments or procedures to reduce pain or distress in those animals that must be used.

Refinements in anesthesia, surgery, analgesia, and many procedures occur frequently.

Examples of refinements include

- ✓ **New anesthetics** that allow rapid induction and reduced recovery times.
- ✓ **New analgesics** that provide more extended pain relief postoperatively with less frequent administrations.
- ✓ **New bleeding and injection techniques** that cause less tissue damage or distress.
- ✓ **Improved surgical techniques** that minimize trauma and the length of anesthesia.

*Answer questions 8-12 on the quiz.*

Surgery on animals requires highly trained, conscientious individuals, and appropriate prior planning. To understand the issues involved, some important terms and concepts must be addressed.

- ✓ **Sterile or Aseptic Technique.** This refers to a series of practices followed to prevent the contamination of the surgical site by microbes during surgery. **If an animal will recover from surgery, sterile technique must be used.**
- ✓ **General anesthesia.** Simply stated, general anesthesia is a state of unconsciousness characterized by a complete lack of pain and sensory perception. **Prior to beginning surgery, you must ensure that the animal will not feel pain during the procedure.** General or regional anesthesia must be provided.
- ✓ **Survival surgery** is surgery in which the animal regains consciousness after anesthesia. If an animal undergoes survival surgery as part of a biomedical research activity, **aseptic (sterile) technique must be used** to prevent postoperative infections, **no matter what vertebrate species is involved.** The **incision site** must be properly prepared prior to the incision.

*Answer question 13 on the quiz.*

Euthanasia literally means a "good death." A more appropriate simple definition is a "gentle death." The USDA **AWA** defines euthanasia as "the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The *Guide* defines **euthanasia simply as "the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress."** Because it is necessary to euthanize most animals as part of experimental protocols, it is very important to use appropriate euthanasia techniques. The technique should result in rapid loss of consciousness followed by cardiac or respiratory arrest and, ultimately, a loss of brain function. In addition, animal handling and the euthanasia technique should minimize distress experienced by the animal prior to loss of consciousness (AVMA Guidelines for the Euthanasia of Animals: 2013 edition, page 7).

Because improper technique can cause pain and suffering to animals during euthanasia, you must be

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trained to properly and humanely perform euthanasia. Of course, the IACUC is interested in your training and ability to humanely perform any procedure on animals, but proper training for euthanasia is an area of emphasis because of the increased potential for harm to animals. Do not perform euthanasia, or any other procedure on an animal until a person experienced with the procedure has trained you, and you feel confident performing the technique. PHS Policy and the *Guide* state that methods of euthanasia should be consistent with the recommendations of a panel sponsored by the American Veterinary Medical Association (AVMA), unless the IACUC approves deviations for scientific reasons. The *AVMA Guidelines for the Euthanasia of Animals* contains many guidelines used by IACUCs to evaluate methods of euthanasia.

To begin, it is important to know that euthanasia methods can be broadly separated into **physical** and **nonphysical (or pharmacologic)** methods.

- ✓ **Physical methods** rely on trauma to the head or spine or fatal loss of blood to cause rapid death. Examples include cervical dislocation, decapitation, captive bolt pistols, and exsanguination ("bleeding an animal out").
- ✓ **Non-physical or pharmacologic methods** rely on drugs to cause loss of consciousness and death.

The AVMA Guidelines classify euthanasia methods as 1) acceptable, 2) acceptable with conditions, and 3) unacceptable:

1. **Acceptable** methods are those that consistently produce a humane death when used as the sole means of euthanasia.
2. Methods **acceptable with conditions** are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met.
3. **Unacceptable** techniques are those methods deemed inhumane under any conditions or that were found to pose a substantial risk to the human applying the technique. These include strychnine, nicotine, caffeine, cleaning agents, pesticides, solvents, and other toxicants not specifically designed for therapeutic or euthanasia use as euthanasia agents under any circumstances.

The AVMA Guidelines also include information about **adjunctive methods**, which are those that should not be used as a sole method of euthanasia, but that can be used in conjunction with other methods to bring about euthanasia.

*Answer questions 14-15 on the quiz.*

If you observe misuse or mistreatment of animals, or you see procedures that you do not think comply with federal regulations or guidelines, report it immediately to your IACUC chair, Dr. Harper. Once an allegation of mistreatment, misuse, or noncompliance is received, the USDA Animal Welfare Regulations and Standards and PHS Policy require the IACUC to review and if warranted, investigate the allegations, whether made by the public or an employee of the institution. The IACUC then makes recommendations to the "Institutional Official" at the institution, who reviews the report and decides if additional action is needed. If the IACUC decides that any animal activities must be stopped to protect either animals or people, they are given clear regulatory authority to do so. In fact, if an IACUC votes to suspend a protocol or animal activity, that decision cannot be overturned by any administrator at the institution. If you see that animals are in danger or in pain, take immediate steps to remove animals from the threat and notify your professor or TA immediately.

*Answer questions 16-17 on the quiz.*

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1. The IACUC's activities to ensure compliance benefit whom?
  - A. The institution, the investigators, and the animals.
  - B. The institution and regulatory agencies only.
  - C. Regulatory agencies only.
  - D. Animals Only.
2. The term IACUC stands for what?
  - A. Institutional Animal Care and Use Committee.
  - B. Institutional Animal Control and Use Committee.
  - C. Institutional Animal Care and Utilization Corps.
  - D. Institutional Animal Control and Utilization Corps.
3. Which of the following "Guides" is best used as a source of guidelines for animals used in food and fiber research and teaching?
  - A. The "Guide for the Care and Use of Laboratory Animals."
  - B. The "Guide to Proper Procedures in Research Facilities."
  - C. The "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching."
  - D. The "Guide to Animal Facility Design and Operation."
4. The USDA AWA regulations do not currently apply to which of the following animals?
  - A. Non-vertebrates, laboratory mice, and laboratory rats.
  - B. Dogs.
  - C. Hamsters and Gerbils.
  - D. Dogs, pigs, and sheep.
5. Explaining the potential value of proposed animal research to human and animal health is
  - A. Important to convey so that everyone on the IACUC, including the lay members, can understand.
  - B. Futile given the uncertain outcomes of scientific experimentation.
  - C. Not necessary because all the members of the IACUC are scientists who will immediately understand the importance of the work.
  - D. Best accomplished using highly technical language that colleagues in your discipline will understand.
6. Which of the following statements concerning selection of species for research is false?
  - A. Vertebrates should be used instead of invertebrates whenever possible.
  - B. Rodents are often selected because of ability to produce genetic modifications in them.
  - C. The most appropriate species should be used.
  - D. A species is often chosen because a disease model exists for it.
7. Which of the following justifications for using a particular species would not likely be accepted by the IACUC?
  - A. Reluctance to learn about a new species that might be a better fit.
  - B. Physiologic characteristics of the species that render it uniquely suited for the proposed research.
  - C. The existence of a large body of background data in the literature for that particular species.
  - D. The availability of species-specific laboratory reagents needed to perform experiments.
8. The three Rs of Russell and Burch stand for what?
  - A. Replacement, reduction, and refinement.

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- B. Replace, recognize, and recover.
  - C. Research, results, and review.
  - D. Research, refinement, and recovery.
9. What is the primary purpose of the "3Rs" concept of Russell and Burch?
- A. Urge scientists to minimize use of animals and to minimize pain and distress caused by animal experiments.
  - B. Provide a reasonable strategy for searching databases to uncover as many plausible alternatives as possible.
  - C. Provide researchers with a means of controlling per diem costs and technical support charges in the animal facility.
  - D. Urge scientists to perform computerized database searches so the institution can prove that alternatives are not available.
10. Which of the following is not an example of replacement?
- A. The use of a new analgesic that provides better postoperative pain relief.
  - B. The use of computer modeling software to replace the use of animals in pharmacologic studies.
  - C. The use of a kit to assay hormone levels instead of a bioassay involving animals.
  - D. The use of cell culture instead of animals to grow immortal cell lines.
11. Which of the following is not an example of reduction?
- A. The use of a new anesthetic that provides better induction and a shorter recovery period.
  - B. The use of improved assays that provide better data precision so that fewer animals are needed per group.
  - C. The use of common control groups during simultaneous experiments to limit the number of control animals needed.
  - D. The shared use of tissues by more than one investigator to minimize animal use.
12. Which of the following is not an example of refinement?
- A. The use of computer modeling software to substitute for animal experiments.
  - B. The use of a new bleeding technique that causes less pain and distress.
  - C. The use of a new analgesic that provides better postoperative pain relief.
  - D. The use of newer surgical techniques that result in less tissue trauma.
13. Which statement does not apply to experimental surgery on animals?
- A. Sterile technique is optional for most survival surgeries.
  - B. A goal of sterile technique is to prevent contamination of the surgical site.
  - C. Either general or regional anesthesia is required to prevent pain.
  - D. Prior planning is very important.
14. Why is proper training in euthanasia techniques so important?
- A. Improper technique can cause unnecessary pain and distress in animals.
  - B. Experiments can be performed more quickly if proper technique is used.
  - C. Euthanasia solutions are expensive and would be wasted if used improperly.
  - D. Training in euthanasia techniques can result in rapid job promotions.
15. Which of the following is a reasonable definition of euthanasia?
- A. The act of killing animals by methods that induce rapid unconsciousness and death without pain or distress.
  - B. The act of killing animals by methods that preserve physiologic function after death.
  - C. The act of preparing animals for a surgical or otherwise invasive procedure.
  - D. The emotional state of humans or animals under distress.
16. If you believe that an animal protocol is not being followed correctly and animals are not treated well,

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who should you tell?

- A. Dr. Harper
- B. Dr. Pie
- C. Dr. Habgood
- D. Dr. St. John

17. Instead of putting an adult fish into the appropriate fish tank water, one of your peers put the fish into tap water. The fish is clearly distressed and struggling to escape the chlorine in the water. What should you do?
- A. Immediately move the fish into appropriate tank water and then tell Dr. Walsh.
  - B. Tell Dr. Walsh.
  - C. Ignore the problem and hope that the fish adjusts.
  - D. Tell your peer to move the fish.