

## Research with Animals Module-2: Animal Care

### 1. Introduction

Module-1 of Research with Animals discussed the basic ethical and regulatory requirements of using animals as research subjects. That module provided information on regulatory requirements in a number of countries, including those of the U.S. and EU. As stated in Module-1, the use of animals for research is considered a privilege that must be conducted in accordance with the highest standards of ethics and care for the animals, as well as those set by the country-specific regulations and the Institutional Animal Care and Use Committee (IACUC) or the Animal Ethics Committee (AEC).

This module provides additional information for individuals who are engaged in performing laboratory animal surgery or other procedures, and for those who are responsible for the administration of anesthetics, or post-procedural care for animals. The specific requirements of each research protocol will be based on the country specific regulations, institutional policies, and the details provided in the protocol submitted to the IACUC, or AEC, for approval.

### 2. Definitions

The following are some important definitions of the terms used in this module:

- a. *Distress*: Distress differs from stress (see below). Distress is most commonly defined as "*a state in which an animal cannot escape from or adapt to the external or internal stressors or conditions it experiences resulting in negative effects upon its well-being*". Principle IV of the US Government Principles states that unless the contrary is established, the assumption must be made that a procedure that causes pain or distress in a human being will cause pain or distress in an animal.
- b. *IACUC or AEC*: Institutional Animal Use and Care Committee (IACUC), or Animal Ethics Committee (AEC), are regulatory mandated committees charged with the oversight of animal care and use at the institution. All research protocols must be submitted to an IACUC, or AEC, for their review and approval before the research may start. Once approved, investigators must follow the protocol as written and any variance or changes must be submitted for review and approval by the IACUC, or AEC, before they can be implemented.
- c. *Major Surgery (or major operative procedure)*: Any surgical intervention that penetrates and exposes a body cavity or any procedure which produces substantial or permanent impairment of physical or physiological functions. Multiple major survival surgeries require special justification in the IACUC protocol application.
- d. *Pain*: It is sometimes difficult to know how animals experience pain and distress. Most regulations and guides define a painful procedure in an animal as: "*any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures.*"
- e. *Protocol*: Detailed description of the research project submitted to the IACUC, or AEC, for its review and approval.
- f. *Survival Surgery*: Any surgical procedure from which the animal is intended to recover consciousness. Aseptic technique must be used for all survival surgical procedures.

- g. *Aseptic Technique*: Surgical technique that is free from infection or septic matter; sterile.
- h. *Non-Survival (Terminal) Surgery*: Any surgery or procedure conducted on animals that are not allowed to regain consciousness. If approved by IACUC in advance, clean (not sterile) procedures may be sufficient for this type of surgery.
- i. *Stress*: A physiological reaction that can lead to an adaptive response.

### 3. Basic Principles

The conduct of any research involving the use of animals must be in accordance with the approved protocol and any deviations from the approved protocol must be submitted for review and approval before they are implemented. It is important that all members of the research team not only be adequately trained in the procedures that they will be involved in, but also be fully familiar with the specific details of all procedures described in the protocol so that they follow them fully.

#### a. *Surgical Procedures*

If you will be conducting survival surgery and anesthesia, remember these four basic principles:

- i. Anesthesia must be appropriate to obliterate sensation without endangering animal health and safety.
- ii. Aseptic technique is required for survival surgery.
- iii. Post-procedural care includes animal health monitoring and appropriate use of post-procedural painkillers and analgesics.
- iv. Documentation of pain management and animal care is required.

***Locations of all surgery areas must be included in the protocol and approved by the IACUC.***

#### b. *Anesthesia*

- i. Anesthetic level must be sufficiently deep so that the animal is unconscious and incapable of feeling pain.
- ii. All anesthetics have potential side effects.
- iii. Anesthetic level must not be so deep that the animal's health is impacted or animal recovery from anesthetic is excessive.
- iv. The anesthetic regimen (i.e. drug and dosage) used must be what is approved by the IACUC.
- v. Anesthesia monitoring: Depth of anesthesia must be monitored and assessed throughout the procedure, to assure that:
  1. The animal is sufficiently anesthetized so as not to experience pain or discomfort.
  2. Anesthesia is not so deep that it could cause harm or death.
- vi. Some anesthesia monitoring parameters require specialized equipment while others require only careful attention to the animal.
- vii. Your IACUC protocol must outline the parameters you will monitor and the frequency with which you will monitor and document them. This **MUST** be followed precisely as written.

***Note: Animals may not be left unattended during a surgical procedure!***

*c. Anesthesia Monitoring:*

Detailed anesthesia monitoring records should be kept for each procedure. These are generally reviewed during all IACUC protocol audits. Anesthesia monitoring and documentation is one of the most important functions during surgical procedures and their inadequacy is often a major reason for citations by the regulatory agencies. ***Please review your protocol and make sure you follow the commitments made!***

Minimum standards for animal anesthesia monitoring include checking for:

- i. Responsiveness to noxious stimuli*  
Responsiveness should be carefully checked before the surgical procedure is begun. Use a foot pinch to assess the depth of anesthesia; for small rodents, a gentle pinch of the animal's foot is a better test of responsiveness than is a tail pinch. Continue observing for responsiveness throughout the procedure. Alternatively and for most species, gently brushing the eyelids is useful. If the animal blinks, it is too light and needs more anesthetic before making an incision. Note that this is not to be confused with the corneal reflex, i.e., touching the cornea to elicit a blink – the cornea is easily damaged and should not be touched unless it is involved in the surgical procedure.
- ii. Respiratory pattern:* Observe the animal's respiratory pattern throughout the procedure. It is very difficult to count a rodent or bird's respiratory rate, but qualitative assessment of pattern and of increased rate can help you identify when an anesthetic's effect is decreasing and additional anesthesia must be provided. Some anesthetics may cause a rodent to stop breathing if the dose is too high. If this happens, turn off the flow of anesthetic (if using inhalants) and cup the animal in the hand and stimulate respiratory chest movements.
- iii. Skin or mucous membrane color:* An animal with depressed respiratory or cardiac function may visibly be either pale or cyanotic (bluish-grey mucous membranes and hairless skin). Watch color throughout the procedure and consider stimulation or administration of oxygen while reducing the flow of anesthetic (if using inhalants).
- iv. Heart rate:* While the heart rate of larger animals is easy to monitor using a stethoscope, it is difficult to count in small rodents. Depressed heart rate may indicate excess anesthesia, while an increase can represent insufficient anesthesia. *Many factors can influence heart rate, and it is rarely appropriate to base anesthetic decisions solely on heart rate without assessing the whole animal.*
- v. Body Temperature:* Animals should be kept as close to normal body temperature as possible, except in rare circumstances where hypothermia is intentionally induced. A heated water pad under the animal is a good means to maintain body temperature.
- vi. Tissue Oxygenation using Pulse Oximetry:* A pulse oximeter is a valuable tool, and there are commercial pulse oximeters that will work on mouse-sized animals. A pulse oximeter will measure heart rate and oxygen saturation, and can be set with alarms to alert the anesthetist of abnormal findings.

*d. Supportive Care during Anesthesia:*

It is important to provide the animals with supportive care while under anesthesia. The specifics of the care provided will depend on the procedure being performed; the length of the procedure and of the anesthetic's effects; the choice of anesthetic; and the species. You should

consult with a veterinarian when planning your study, and consider providing supplemental warmth; supplemental oxygen; intravenous, subcutaneous or intraperitoneal fluids, as appropriate or recommended by the veterinarian.

#### 4. Aseptic Surgical Technique

The goal of asepsis is to prevent an infection that could adversely affect an animal's well-being or healing. An infection could adversely impact the outcome of your research and render the results useless. Therefore, all surgical procedures must be conducted under aseptic conditions, unless the IACUC or AEC has approved otherwise (e.g. for terminal surgeries). There is no single procedure, piece of equipment, or germicide that can achieve asepsis on its own; but using a comprehensive infection control approach could prevent it.

The following are the general guidelines for common practices used in preventing infections during surgical procedures. The risk of surgical infections may increase depending on:

- a. The length of the procedure and whether tissue is handled gently and kept moist, or handled roughly and allowed to desiccate. *Note: Sharp scissors will cause less crushing injury than dull ones do -- use the best quality surgical instruments and keep them in good repair.*
- b. Incision size (larger incisions may increase the risk of contamination).
- c. The sterility and materials of any implants left in the body (pumps, suture, cannulas, etc.).
- d. The immune status or genetic make-up of the animal.
- e. As a general rule, aseptic technique for major survival surgery in animals requires:
  - i. A facility that separates a sterile surgical area from animal housing as well as animal and surgeon preparation area.
  - ii. Removal of fur and feathers from the surgical site for skin disinfection, followed by proper skin disinfection of the surgical site (after anesthesia is induced).
  - iii. Those performing surgery should don face mask, shoe covers, hair cover and then scrub their hands and forearms with disinfectant using proper technique.
  - iv. A sterile gown and sterile gloves are then donned by surgeons, who then must drape the surgical field with sterile towels, being careful not to contaminate the field by touching non-sterile surfaces. Sterile gloves must be replaced immediately if non-sterile surfaces are touched.
  - v. Others in the room should wear appropriate protective garb (head cover, face mask).
- f. Use of sterile supplies and instruments. Sterilizing supplies usually requires either steam-sterilization in an autoclave, ethylene oxide gas sterilization, or an approved chemical sterilization technique. Supplies must:
  - i. Have the date of sterilization written on them.
  - ii. Unless specifically labeled with an expiration date, these must be discarded or re-sterilized if they exceed recommended shelf-life. Most common shelf lives include: cloth-double wrapped - 1 month; paper/plastic peel packages - 6 months to 1 year; airtight plastic to cover linens - 6 months.

*Note: in most jurisdictions the use of expired materials such as sterile solutions, equipment, anesthetics, or analgesics for surgical procedures is strictly forbidden!*

## 5. Post-surgical Care

Post-surgical care is the support provided for the animals during the immediate post-operative period until the animal has recovered enough with stable vital signs to take care of itself again (e.g. be able to right itself if laid on its side, feed itself, etc.). The duration of the post-surgical care will depend on the procedure performed and the speed of recovery. This period may be extended in cases where complications have occurred or a physical impairment has been induced. During this time period, routine (e.g. every X hours or daily) recorded observations are required. The protocol will include a specified period of time and care required, although a veterinarian may extend the time based on the observations made during post-surgical examination. The following are some principles of adequate post-operative care:

- a. Your protocol's monitoring commitments must be followed EXACTLY as approved - this may require attending to animals through the night on the first day or two after a major procedure.
- b. After surgery, anesthetized animals should never be left alone until they have regained righting reflexes and can sit upright in the cage.
- c. Always document the time that righting reflexes have returned after any anesthetic episode.
- d. Painkillers (analgesics) should be initiated while the animal is still anesthetized.
- e. During the convalescent days following the surgery, ensure that animals are keeping their sutured (or clipped) surgical incision and bandage clean and intact.
- f. Monitor progress during the convalescent period, including food and water consumption, body weight, etc. as described in the IACUC protocol.
- g. Assess pain and administer analgesics at the dose and frequency listed in the animal protocol. Remember that some anesthetics (such as isoflurane vapor) have minimal residual pain-relief once the animals are awake. Assessing animal pain can be difficult. How an animal exhibits pain will depend on the species, the individual animal, and the cause and type of pain. Some of the parameters to consider in your pain assessment:
  - i. Decreased activity level.
  - ii. Decreased food or water consumption.
  - iii. Loss of body weight.
  - iv. Limping or other disuse of body parts.
  - v. Biting or scratching at surgical incision site or bandage.
- h. If your protocol says you will assess pain and administer analgesics:
  - i. Every 8-12 hours, you must do it within that time frame after surgery is completed including evenings, nights, or weekends.
  - ii. "As needed" or "PRN," there MUST be a written note that you assessed pain at the required frequency. For small rodents, a group note in the laboratory notebook may suffice.

## 6. Documentation of Surgery and Anesthesia

Maintaining proper and complete documentation of all surgical and anesthesia monitoring and assessments is extremely important. In general, the regulatory agencies follow a simple rule during their inspections: "if it is not written, it was not done." Things to keep in mind when doing your research:

- a. It is best that every time you visit the animal room for observation, you make a note in the records of your presence and observations. You can never over-document.
- b. RECORD ALL actions taken, add time, date, and your name.
- c. Document medications, anesthetics, analgesics used.
- d. Review your animal use protocol. If there is a commitment to using a particular medication, you must record its use, no matter how routine it may seem.
- e. Your protocol contains commitments for intra-operative and other monitoring of your animals, including frequency of monitoring (e.g., “Heart rate every fifteen minutes” or “Signs of pain daily.”). If these commitments are in your protocol, the documentation should be in the animal’s records.
- f. Adhere to the monitoring commitments and schedule in commitments in your approved protocol. For example, if you have committed to:
  - i. Monitoring the animals every 15 minutes until they are feeding, there must be written records of the monitoring and the time the animal started taking food.
  - ii. Checking an animal every 2 hours for the first 48 hours, there should be documentation of 24 visits.
- g. When a group of small animals is treated identically, individual identification and records may not be necessary. For example:
  - i. It can be acceptable to record that all animals in 3 cages received a standard anesthetic dose for a surgical procedure.
  - ii. The next day, record that all the animals were examined for signs of pain, treated with a pain-killer, and are in good condition.
  - iii. Individual identification and records sometimes are required for group-housed rodents. If a protocol calls for monitoring body weight, for example, this can only be accomplished if animals are individually identified.

## 7. Veterinary Communications

Regulatory frameworks require that research institutions appoint a veterinarian who has knowledge of laboratory animals and who will advise the IACUC or AEC and researchers on the proper care and use of animals. It is important that researchers maintain a relationship with the veterinarians in order to establish routine communication with them. Good communication with the veterinarians not only contributes towards better animal care, but it also ensures that the study is conducted in compliance with the regulatory requirements. The following are a few examples of when researchers need to communicate with the veterinarians:

- a. Performing survival or prolonged procedures on animals actively under veterinary care without contacting the veterinarian(s) first.
- b. If an animal has had a medical condition identified, or treatment has been prescribed, and the veterinarian has not made it clear whether that animal must be restricted from research use, call the vet. Write in the record: “Acceptable for procedure per Dr. \_\_\_\_\_”
- c. Administering medications or treatments not in your protocol. If an animal requires antibiotics or other treatments above or beyond what is in your protocol, they should be administered on the orders of a veterinarian.
- d. The veterinarian should chart this prescription in the record, or you should write: “Drug and dose started per consultation with Dr. \_\_\_\_\_”

## 8. Euthanasia

Research animals are normally euthanized at the end of a study for the purpose of sample collection or post-mortem examination. Animals may also be euthanized if they are experiencing excessive pain or distress that cannot be alleviated by medication, unless allowed in the protocol by the IACUC, or if a veterinarian deems the animal's pain unacceptable or inappropriate and exceeds what was approved by the IACUC. Euthanasia must be conducted in humane manner, using methods that induce rapid unconsciousness and result in animal death without pain or distress.

The protocol approved by the IACUC or AEC includes the criteria for when and how an animal will be euthanized. The researchers must follow the methodology approved by the IACUC or AEC and the individuals who perform the euthanasia must be trained and approved by the IACUC or AEC.

AVMA Guidelines for the Euthanasia of Animals is an excellent source of information regarding various humane methods of euthanasia for various species.

## 9. Final Thoughts

Humane use of animals in research is a moral and ethical duty of all individuals engaged in such research. As discussed in Module-1 and in this module, while there are variations in the specific regulations governing the use of animals in research, there are universal principles that are common to all. This module has provided basic principles of good research practices in working with animals. Researchers engaged in animal subject research must make sure they understand the specific regulatory framework governing animal research in their country. Researchers must also make sure that all individuals participating in their research are adequately trained not only in ethical and regulatory issues, but also in the experimental procedures and proper care of animals. Additional training should be sought for specific procedures (e.g. handling of animals; surgical techniques; administration of anesthesia; monitoring of animals under anesthesia; assessment of pain and distress; euthanasia techniques) for which the research team may not have experience or expertise in. The need for training is more important for trainees and young scientists who may not yet have adequate hands-on experience through their work.

An important part of the process to remember is the submittal of a detailed protocol to the IACUC or AEC describing the research conducted and procedures performed. Of most importance is following the approved protocol as it is written. Researchers should also maintain communication and transparency with the institution's laboratory animal veterinarian, who will provide guidance and support. Good procedures and aseptic techniques, as well as proper care during and after surgery, will ensure the welfare and health of animals and will contribute towards good outcomes for the research.

## RESOURCES

- U.S. Animal and Plant Health Inspection Service (APHIS)  
<http://www.aphis.usda.gov>

- U.S. Animal Welfare Act as Amended (7 USC, 2131-2156)  
<http://www.nal.usda.gov/awic/legislat/awa.htm>
- Health Research Extension Act of 1985: *Animals in Research*  
<http://grants.nih.gov/grants/olaw/references/hrea1985.htm>
- U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals  
<http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf>
- National Research Council Guide for the Care and Use of Laboratory Animals  
<http://www.nap.edu/readingroom/books/labrats/>
- American College of Laboratory Animal Medicine (ACLAM) (1996): Adequate Veterinary Care.  
[https://www.aclam.org/Content/files/files/Public/Active/position\\_adeqvetcare.pdf](https://www.aclam.org/Content/files/files/Public/Active/position_adeqvetcare.pdf)
- NIH Office of Laboratory Animal Welfare (OLAW)  
<http://grants.nih.gov/grants/olaw/olaw.htm>
- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training  
<http://grants.nih.gov/grants/olaw/tutorial/relevant.htm#2b>
- Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)  
<http://www.aaalac.org>
- DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes  
<http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&from=EN>
- United Kingdom Guidance Research and testing using animals  
<https://www.gov.uk/research-and-testing-using-animals>
- Australian code of practice for the care and use of animals for scientific purposes 7th edition (2004)  
<https://www.nhmrc.gov.au/guidelines-publications/ea16>
- Canadian Council on Animal Care (CCAC)  
[http://www.ccac.ca/en/\\_standards](http://www.ccac.ca/en/_standards)
- The American Veterinary Medical Association (AVMA)

[https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx?utm\\_source=prettyurl&utm\\_medium=web&utm\\_campaign=redirect&utm\\_term=issues-animal\\_welfare-euthanasia-pdf](https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx?utm_source=prettyurl&utm_medium=web&utm_campaign=redirect&utm_term=issues-animal_welfare-euthanasia-pdf)