

## **SUPPLEMENTAL INSTRUCTIONS for the ANIMAL PROTOCOL REVIEW FORM**

Institutional Animal Care and Use Committee  
Ross University School of Veterinary Medicine

### **IACUC ANIMAL PROTOCOL approval cannot be given until the following are submitted:**

1. A complete Animal Protocol Review Form;
2. Form 12. Adverse events and early ending of the study;
3. As applicable, the additional forms listed in Section 11 of the Animal Protocol Review Form; and
4. All approvals from other committees, when required, in regards to human and environmental health and safety and the use of biological, chemical or radioactive substances and recombinant DNA.

**The following instructions are meant to provide supplemental guidance for completion of the Animal Protocol Review Form and Forms 1-12 listed in Section 11 of the Animal Protocol.**

### **Section 1. General Information**

#### **1 A&B. PI and secondary contact**

- Principal Investigator (PI) - the person responsible for the project/course coordinator.
- Secondary contact person - a secondary person should be assigned to ensure there is always someone available to answer questions pre-, during and post-use of animals if the PI is unavailable.

#### **1C. General protocol information**

- Title - maximum of 95 characters (including spaces); the title should be short but descriptive.
- Type of protocol – new (new and renewals); continuation (protocol for which the time period has been extended).
- Type of project – research (investigative); teaching (for a class or club); pilot (applicable to research; must clearly state the benefit of a pilot study in Section 2A how).

#### **1D. Necessary approvals**

- In all cases of “No” ensure that it is clear why it has not been done or that it will be done prior to animal use.
- In all cases of “N/A” ensure that it is clear why it is not applicable (in the project narrative Section 2C).

#### **1E. Animal information**

- Species – feline, canine, ovine, etc.
- Strain – indicate if a specific breed or genetic modification is required.
- Special requirements – indicate if a specific sex, disease infection, etc. is required.
- Sourced internally – most RUSVM animals are used in multiple protocols; ensure in discussions with animal resources/ other responsible parties that there is a clear understanding of other uses for the animals and that rest periods are met.
- Externally sourced -- indicate from whom they will be purchased and how they will be transported in the project narrative (Section 2C).

**Section 2. Animal Use and Procedures****2A. Purpose and scientific benefit**

If the research is duplicative, the benefit of repeating the work must be explained. If a pilot study, indicate how the data will be used.

**2B. Flow diagram**

Example: Effect of Postsurgical Radiation on the Bowel

Group 1. 96 mice (8 animals/test group X 6 different doses of radiation x 2 sites of int. adhesions)

Day 1 Surgery to create adhesions and radiation to the abdomen under pentobarbital anesthesia.

Day 1-100 Monitored daily for clinical signs of intestinal obstruction and/or radiation disease.

Group 2. 96 mice (8 animals/test group X 6 different doses x 2 sites of int. adhesions)

Day 1 Radiation to abdomen, under anesthesia.

Day 1-100 Monitored daily for clinical signs of radiation disease.

Group 3. 16 mice (8 animals/test group X 2 sites of int. adhesions)

Day 1 Surgery to create adhesions, under anesthesia.

Day 1-100 Monitored daily for clinical signs of intestinal obstruction.

All Groups (1-3)

Day 1-100 Moribund animals or animals in pain will be euthanized.

Day 100 Euthanized and tissues taken for assessment.

**2C. Project narrative**

All instructions included in the Animal Protocol Review Form

**2D. Veterinary care**

Ensure veterinary care is available 24/7 for emergencies and over semester break periods and when the PI is off-island.

**Sections 3-6:** All instructions included in the Animal Protocol Review Form

**Section 7. Rationale**

The rationale must include alternatives to the use of animals considered and reasons for not using alternatives to animals. Animal numbers should be justified statistically. Alternatively, if regulatory guidelines are available, the number required by the guidelines can be used as a justification. If animals are being used for teaching, an explanation regarding the number of animals selected in relation to the number of students should be provided and ensure animal numbers match what is stated in the syllabus and Section 2E.

**Section 8. Literature Review**

If the research is duplicative or alternatives are found and not used, a statement explaining the rationale must be in the project purpose or narrative (Section 2A or 2C), the rationale (Section 7) or appended to the protocol.

**Section 9. Personnel Qualifications and Training**

Ensure that responsibilities listed here match those in Forms 1-12.

In cases where participating personnel are unable to complete CITI IACUC training provide an

assurance statement that equivalent training will be provided and documented. In cases where participating personnel are not yet identified, indicate how they will be included in the protocol (e.g., by amendment).

**Section 10. Teaching Syllabus/Materials:** All instructions included in the Animal Protocol Review Form

**Section 11. Additional Forms:** see instructions for additional forms below

### Principal Investigator Assurance Statement

Once the protocol is approved and this statement is signed, any planned changes to the protocol described on this form or any of the additional forms must be submitted to the IACUC as an amendment and approved by the IACUC prior to implementation. If a deviation does occur, it must be documented and reported to the IACUC. All AEs also must be reported. Standard forms for Amendments, Deviations and AEs are available.

### Instructions for additional forms (Forms 1-12)

#### Form 1. Euthanasia

Acceptable methods of euthanasia for various species and sizes of animals are outlined in the Report of the AVMA Panel on Euthanasia (<https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx>) and in RUSVM IACUC SOPs. The AV must be consulted for all studies that require death as an endpoint.

#### Form 2. Exogenous substance administration

Tumor Burdens should not be greater than 10% of the bodyweight unless scientifically justified. Animals must be closely monitored.

Repeated Injections of Complete Freund's Adjuvant are discouraged. It is suggested that you explore the use of other adjuvant systems or that you use Incomplete Freund's for boosters.

Intracardiac Injections must be performed by trained personnel in anesthetized animals.

#### Form 3. Samples collected from a live animal

This form does not need to be completed if fluids will be extracted from an anesthetized animal that will not regain consciousness. In all other cases of fluid extraction (e.g., blood, urine, ascitic fluid, CSF, joint fluid, etc.) and tissue/sample collection, this form must be completed.

Safe blood sample volumes must be no more than a maximum of 7.5% of the Total Blood Volume on repeated weekly draws or 15% of the Total Blood Volume on a single occasion. Administering saline intravenously to replace the lost blood volume will minimize adverse effects.

Estimated Total Blood Volumes of Animals (ml/kg bodyweight)

Rat	64 ml/kg	Rabbit	56 ml/kg	Cat	75 ml/kg
Mouse	78 ml/kg	Rhesus	75 ml/kg	Pig	65 ml/kg

Guinea pig	75 ml/kg	Dog	86 ml/kg	Sheep	60 ml/kg
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For animals not listed, consult with the AV to determine a maximum quantity.

*Source: A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Vectors. J. Appl. Toxicol., Diehl*

Withdrawal of blood by cardiac puncture must be performed by trained personnel and only on animals that have been anesthetized.

Rodent retro-orbital bleeding requires anesthesia. Sterile plastic tubing, cut on a bevel, is recommended. Glass tubing, if used, should have polished ends

Hybridoma plus ascitic fluid should not become greater than 10% of body weight. Animals should be closely monitored and euthanized if they become ill. One or two harvests prior to euthanasia is strongly encouraged.

#### **Form 4. Anesthesia/analgesia /muscle-paralyzing agents**

A full anesthesia record must be kept including dose and drugs administered. This is especially critical when the PI is not administering the anesthesia/analgesia /muscle-paralyzing agents. PI staff includes, but is not limited to the anesthesiologist (when not the PI), students and/or technicians. The PI is always ultimately responsible that correct procedures are followed.

Anesthetics/analgesics reference charts can be requested from the IACUC or AV.

Muscle paralyzing agents must only be used in animals that are anesthetized.

#### **Form 5. Surgery or major procedure**

PI staff includes, but is not limited to the anesthesiologist (when not the PI), students and/or technicians. The PI is always ultimately responsible that correct procedures are followed and that all involved personnel are trained in the procedures.

Non-Survival Surgery is a surgical procedure in which the animal does not regain consciousness. Survival Surgery is a surgical procedure in which the animal regains consciousness.

A Major Procedure is any survival procedure, other than surgery, which produces permanent impairment of physical or physiological functions. Such procedures would include radiation or rendering an animal diabetic.

In general, it is required that surgeries are performed in an IACUC approved aseptic facility. Some exceptions are permitted. Nonsurvival surgery may be performed under less strict conditions, but the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean.

Intraoperative care and monitoring must be provided for all animals undergoing surgery, including nonsurvival surgery.

Anesthesia recovery should include support and monitoring of vital functions and level of analgesia.

At a minimum, clinical recovery should include daily monitoring for 3-5 days after surgery. Abnormal clinical findings should be reported to the veterinary staff and appropriate care

provided.

**Form 6. Potential for pain /distress/discomfort, more than momentary**

In general, conditions that cause more than momentary pain, distress, or discomfort in humans will have the same adverse effects on animals. Such conditions that may cause pain or discomfort include, but are not limited to: surgery, excessive inflammation, necrosis, drug or radiation toxicity, noxious stimuli without escape, functional impairments. Such conditions that may cause distress include, but are not limited to: restraint of the unadapted animal, restraint for more than 4-8 hours, abnormal diet or environment, aversive conditioning.

Unless scientifically justified, means for alleviating animal pain, distress or discomfort must be instituted. Consultation with the AV is recommended for procedures that might result in pain, distress or more than momentary discomfort.

**Form 7. Special housing, caging, husbandry; minor hunger/thirst**

This form is to be completed if the animals are to be managed in any way other than what is considered to be normal for the species. This includes any special housing or husbandry requirements, if feed or water is to be withheld for any period of time, if any special diets or fluids are to be offered, if special light cycles are required, if there are special temperature requirements or if the animals will be kept outside of the main animal facilities (for that species) for more than 24 hours. In all cases, deviations from the standard care of an animal must be justified, the potential effects on the animal described and methods for monitoring and alleviating adverse effects described. In addition, the program for monitoring the physiologic and behavioral well-being of those animals maintained in any way other than the normal for the species must be described and the criteria for temporary or permanent removal of an animal from the experimental protocol explained.

For non-standard diets, it must also be noted if the diet is or is not nutritionally balanced.

In the case of dietary or water restrictions, state how and why the food and/or water will be restricted, the scientific justification, the degree of restriction in % of the ad libitum or normal daily intake or as a % change in an animal's body weight.

**Form 8. Physical restraint, more than momentary**

Any cage or device that will not allow the animal to make normal postural adjustments is considered a restraint device. Animals can experience stress reactions to prolonged restraint when they have not been properly acclimated. Acclimation should include introduction of the animal to the restraint device, and gradually increasing the time that they are restrained.

Tethering is not considered restraint.

Ruminant metabolic cages are not considered restraining unless the animals are kept in the cage for more than 8 hours at any one time

**Form 9. Behavioral testing**

This form applies to any projects involving behavioral observations or interventions.

**Form 10. Breeding**

When breeding animals, records of crosses and weaning must be maintained.

**Form 11. Uncommon procedures (not adequately described elsewhere)**

Use the space on this form to describe any procedures not adequately described elsewhere and that you believe will assist the IACUC in properly assessing the project.

**Form 12. Adverse events and early ending of the study (REQUIRED FOR ALL PROTOCOLS)**

All studies and courses with live animals have the potential of having adverse events. During a simple physical examination a horse could kick something and injure a leg and a dog can break a dew claw. In addition, even in the most benign uses, an animal, based on temperament/situation/having a bad day, might need to be withdrawn from a teaching protocol or study.