Introduction to Rutgers University IACUC Policy Handbook

Regulations
The Public Health Service (PHS) and AAALAC International (AAALAC) require that institutions comply with the standards in the "Guide for the Care and Use of Laboratory Animals" and the Animal Welfare Act (AWA) regulations, where applicable. The Institutional Animal Care and Use Committee (IACUC) must comply with the national standards and recommendations contained within the Guide and the Animal Welfare Act and Regulations. All exceptions to the Guide standards and recommendations need to be described and justified in an IACUC approved protocol prior to initiation.

AAALAC International Standards
AAALAC expects accredited institutions to comply with all national or regional regulations, policies and guidelines, as well as conditions of funding. Additionally, AAALAC considers performance standards paramount when evaluating care and use of animals in research, testing, or teaching. The performance criteria described in the "Guide for the Care and Use of Laboratory Animals" (the Guide), the "Guide for the Care and Use of Agricultural Animals in Research and Teaching" (Ag Guide), and European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123) are used by AAALAC in assessing standards of care. Where AAALAC guidance in the form of Position Statements is available, the standards described in the Statements will be incorporated into the policies.

Rutgers Standards

- **This document covers the minimum requirements that all investigators at Rutgers must comply with when using animals in research, teaching, testing, or production.**
- **Individual IACUCs may require more stringent oversight or procedures as applicable.**
- **What is written in the protocol will always supersede what is written in the policy until the protocol undergoes review or at the discretion of the IACUC. This means that the protocol stands as written even if a policy has been updated. However, when a triennial review is submitted, the protocol must be updated to reflect the current polices.**
- **Failure to comply with the IACUC Policy Handbook may result in suspension of privileges to work on a protocol.**

Always consult with the IACUC office or a CMR veterinarian for assistance in interpretation and application of IACUC policies.

IACUC office staff and CMR veterinarians are available for protocol preparation assistance and guidance for all procedures. It is particularly important to ask for assistance in writing protocols where animals will experience pain and distress.

Common Acronyms used throughout the Policies

IACUC: Institutional Animal Care and Use Committee

eIACUC: the electronic submission program for protocols to be reviewed by the IACUC

CMR: Comparative Medicine Resources

AV: Attending Veterinarian

Reference Links

Animal Welfare Act
https://www.aphis.usda.gov/animal_welfare/downloads/AC_BlueBook_AWA_FINAL_2017_508comp.pdf

PHS Policy
https://olaw.nih.gov/policies-laws/phs-policy.htm

Guide for the Care and Use of Laboratory Animals, 8th edition
https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf

Guide for the Care and Use of Agricultural Animals in Research and Teaching, 4th edition
https://www.asas.org/services/ag-guide

European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes
https://aaalac.org/about/ETS123.pdf

AAALAC International Resources for Investigators
https://www.aaalac.org/resources/investigatorinfo.cfm

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals

RUTGERS
UNIVERSITY

IACUC Policy Handbook

Policy Table of Contents

G. Husbandry/Facilities:

H. Administrative Polices:

RUTGERS UNIVERSITY IACUC Document #A1	Anesthetics and Analgesics in Laboratory Animals
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 8/18/2021 (version 4.0)

I. Purpose

The purpose of this document is to provide investigators with guidance regarding the most appropriate and most common choice of anesthetic and analgesic agents for experimental procedures across a wide range of species.

II. Introduction

A fundamental responsibility of individuals who use animals in research, teaching or testing is to **anticipate and eliminate or minimize any potential that procedures may cause animal pain, distress, or discomfort**. The specific anesthetic(s) and analgesic(s) that will be used must be specified in the approved protocol. Additional drugs and doses not listed in this policy may still be included in the protocol.

III. Methods

A. General Considerations

1. In order to reduce anesthetic risk and prevent post-anesthetic complications, animals must first be examined for signs of disease or distress including, but not limited to, ruffled, matted or dull hair coat, labored breathing, lack of inquisitiveness, failure to respond to stimuli, abnormal posture/ positioning, dehydration, or impaired locomotion.

2. When planning to administer drugs, recall that **dosage charts** for anesthetic and analgesic agents state only the **recommended amount of drug** that would be expected to produce a desired level of anesthesia or analgesia under standard conditions. Consequently, animals must be monitored carefully and the dosages tailored to meet each clinical and research situation.

3. The duration of anesthesia produced by the anesthetic should coincide with the expected duration of the operative procedure. The duration of analgesia produced by the analgesic should coincide with the expected duration and intensity of post-operative pain generated by the procedure. The time required for post-surgical recovery from anesthesia, as well as the frequency of administration of analgesics should be based on the species, anesthetics used, and the procedure performed. Knowledge, experience and skill with available agents and equipment are essential to the successful use of anesthetics and analgesics.

B. Controlled Substances

1. Many of the drugs described in this guide have the potential for human abuse and must be maintained in a manner consistent with Public Law 91-513.

2. All controlled substances must be requested and obtained from the campus centralized Controlled Substances Program.

C. Pre-Anesthetic Treatments

1. Pigs, cats, dogs, and primates should be fasted for 8-12 hours prior to surgery to minimize the risk of vomiting and aspiration of vomit during induction of anesthesia and during recovery. Since rabbits and rodents do not vomit, they do not require fasting. Fasting ruminants has little effect on the volume of ingesta in the rumen.

2. All animals must be weighed within three days prior to surgery to ensure accurate injectable drug dosage calculations.

3. Drugs such as anticholinergics, tranquilizers, or sedatives are given as anesthetic pre-treatments to minimize stress, anxiety or excitement of the patient, to ease the transition to the first plane(s) of general anesthesia, to decrease the amount of anesthetic agent, to prevent vomiting, and to control salivary and bronchial secretions.

4. Administering a sedative or tranquilizer when the animal is still in its pen or cage and allowing the drug to take effect before moving the animal to the prep area or operating room may significantly reduce the animal's stress.

D. General Anesthetics

1. Anesthesia is the act of providing sensation-free relief from pain or pain-producing procedures. Anesthesia must be performed by a person with knowledge of and familiarity with the drugs to be used in the animal species under consideration. The Principal Investigator must ensure adherence to IACUC-approved procedures during performance of the protocol and is responsible for ensuring that all staff are trained in the proper use of tranquilizers, anesthetics, and analgesics appropriate for the species and planned procedures.

2. Many factors can affect the activity of anesthetics. The species, strain, sex, age, nutritional and disease status, relative body size, disposition/demeanor, presence of concurrent pain or distress, or medication are known to cause a variation in the amount of drug needed to produce a desired effect in an individual animal.

3. Although the mechanisms of action vary, anesthetics produce, in a controllable manner, both loss of consciousness and an absence of motor response to noxious stimuli. Unconsciousness, analgesia, and muscle relaxation should be sufficient to allow the performance of procedures without the subject experiencing pain. In addition to these effects, anesthetics also produce a depressive effect on the cardiovascular, respiratory, and thermo-regulatory systems. Their use must be monitored closely.

4. The level of anesthesia should be limited to the induction of the minimal degree of central nervous system depression necessary for performing the procedure. When an injected anesthetic agent is used, drug dose calculation should be based on body weight. General anesthesia must be given "to effect," as noted in physiologic responses and in response to noxious stimuli. It is important to realize that some drugs take time to take effect. Anesthetic death can be attributed to administering a supplemental dose of anesthetic without allowing for sufficient time to take effect. This is especially true of parentally administered drugs (e.g., barbiturates). Once they are injected, there is little the anesthetist can do to control the outcome.

5. **Inhaled Anesthetics** (isoflurane is the most commonly used agent) have a greater margin of safety and produce a more stable plane of surgical anesthesia when used with a calibrated vaporizer than injectable anesthetics. Since these anesthetics enter and leave the body via the respiratory system, the concentration of the anesthetic in the blood and brain can be changed rapidly, thus readily altering the depth of anesthesia. Elimination of these anesthetics is primarily by the lungs, allowing rapid induction and smooth and rapid recovery.

6. Intubation is the recommended method for administering inhalant agents to non-rodent species. For most rodent species, inhaled anesthetics can be administered by mask. Intubation allows rapid response to hypoventilation or respiratory arrest through mechanical ventilation using the anesthetic machine.

7. Safety precautions should include the protection of humans from vapors of inhalant anesthetics, which can cause reproductive and other health problems. This is best accomplished by the use of an approved gas scavenging system or by using the inhalant anesthetic agent inside an approved fume hood. Intubation eliminates the release of gas into the room air that occurs when a mask is used.

8. **Injected Anesthetics** (e.g., pentobarbital, ketamine) produce a depth of anesthesia that cannot be readily altered. Injectable agents are eliminated by redistribution in the body, liver metabolism, and renal excretion. Recovery from these agents is more dependent on hepatic and renal function as well as body mass and fat than inhaled anesthetics. Animals under injectable anesthesia usually are not intubated and breathe room air. Hence, animal responses to respiratory emergencies are delayed. Despite these drawbacks injectable anesthetics are safe and effective to use in many situations. (Note: In rodents, ketamine must be used in combination with other agents: see tables below.)

E. Neuromuscular Blocking Agents (NMBAs)

1. Neuromuscular blocking agents (immobilizing drugs or paralytics) inhibit the transmission of nerve impulses at the neuromuscular junction (e.g., succinylcholine) or at spinal synapses (e.g., mephenesin, guaifenesin) resulting in skeletal muscle paralysis and profound muscular relaxation without loss of consciousness. These agents are used as an adjunct in surgical anesthesia to obtain more complete muscle relaxation for specific procedures (e.g., bone fracture repair in heavily muscled animals such as horses). Scientific justification must be provided in the IACUC protocol for the use of NMBAs.

2. Depolarizing neuromuscular blocking drugs (e.g., succinylcholine) cannot be reversed. Competitive neuromuscular blocking agents (e.g., d-tubocurarine, pancuronium) can be reversed by administering anticholinesterases (e.g., neostigmine, pyridostigmine). These agents produce muscle paralysis only. They **do not produce sedation or analgesia, and must never be used as an anesthetic or analgesic agent** (9 CFR 2.31: NRC. 1996: PHS. 1996). Since these agents paralyze the muscles of respiration, endotracheal intubation and mechanical ventilation are necessary. Neuromuscular blocking agents, when used in surgical procedures, are restricted to anesthetized animals.

F. Monitoring Anesthesia

1. General anesthesia always carries the risk of compromising the animal's vital functions and even death. Animals should be closely monitored during induction, maintenance, and recovery from general anesthesia. Cardiovascular, respiratory, thermo-regulatory function and depth of anesthesia must be frequently assessed. This requires observation of both vital signs (e.g., heart rate, blood

pressure, respiratory rate and depth, color of mucous membranes, capillary refill time, body temperature) and reflexes (e.g., toe pinch, tail pinch, eyelid/eyelash, palpebral). **Vital signs** are indicators of basic homeostatic functions and **reflexes** help to assess depth of anesthesia. No one parameter is sufficient to assess the effect of anesthesia on an animal. All parameters must be considered in combination to determine the animal's response to anesthesia.

2. **Reflexes** are absent and muscle tone is relaxed during surgical anesthesia. The pedal withdrawal reflex (i.e., toe pinch), eyelid/eyelash reflex, palpebral reflex, and the tone of jaw and anal sphincter muscles can be readily evaluated in larger mammals such as dogs, cats, and pigs. The pedal withdrawal reflex can be used in all species. In rodents, it is recommended to test reflexes by pinching both rear feet and tail. Ocular position and pupillary size are unreliable indicators of depth of anesthesia. However, a widely dilated pupil, with little or no iris visible, should always cause concern, since it may be the result of an excessively deep plane of anesthesia, or hypoxia.

3. **Respiratory Signs** – Anesthetists should monitor the rate, rhythm, and depth of respiration and mucous membrane color. An increase in respiratory depth, regular rhythm, and decrease in respiratory rate signifies surgical anesthesia. Cyanotic mucous membranes indicate hypoxemia from inadequate lung ventilation. Opioids can cause severe respiratory depression, which can be reversed by the administration of naloxone. Respiratory arrest usually precedes cardiovascular collapse.

4. **Cardiovascular Signs** – It is often difficult to manually monitor heart rate in rodents, however the use of monitoring equipment may facilitate successful anesthesia. An increase in rate (tachycardia) during anesthesia often indicates that the depth of anesthesia is not adequate. A decrease of rate (bradycardia) during anesthesia may signify an excessive dose of anesthetic. Opioids, alpha-2 agonists, and vagal reflex activity can cause bradycardia. If the depth of anesthesia can be determined to be appropriate using other parameters, the use of anticholinergics can counteract these effects. Pulse strength, rhythm, and rate are readily determined in larger mammals by digital pressure over an accessible site (e.g., femoral artery, tail artery, auricular artery, lingual artery). Capillary refill time (CRT) is an indicator of peripheral perfusion and is normally less than 2 seconds. During lengthy procedures, anesthetized animals may become dehydrated. To help maintain normal hemodynamics, warm, balanced electrolyte solutions should be administered, by continuous intravenous drip, throughout the surgical procedure. Rodents may be administered fluids via the subcutaneous route.

5. **Body Temperature** – Anesthetics usually cause a depression of body temperature. Body temperature can be measured rectally in most species. Maintaining body temperature at normal levels allows more rapid metabolism of anesthetic agents. To avoid hypothermia, body temperature should be monitored and maintained throughout the anesthetic process and post-operative period. Conservation of body heat is an integral part of anesthetic management. Core body temperature can fall precipitously during general anesthesia, especially in small animals, and when combined with other factors, can lead to delayed anesthetic recovery or death. An external warming source is recommended to help maintain body temperature.

6. **Post-anesthesia** –Monitoring should continue until the animal attains sternal recumbency and exhibits purposeful movement. For non-rodent species, monitoring should continue until normal body temperature is maintained and the animal is able to eat and drink. Some anesthetics and analgesics can affect animals for days after administration. Therefore, it is important to check animals

for signs of anorexia, fever, vomiting, or abnormal respiration or heart rate until the animal resumes normal function.

7. **Indications of Anesthetic Overdose** – Monitoring vital signs continuously during anesthesia will provide early warning of potential problems and emergencies that may be averted by appropriate and quick corrective actions. Do not rely on a single parameter to assess the animal's condition. All parameters should be evaluated prior to initiating any corrective actions.

The following indicators of anesthetic overdose, which may lead to cardiac or respiratory failure, are helpful in assessing the animal's status during anesthesia:

- Heart rate may be rapid or slow, depending on the animal's state of physiological decompensation. Remember that anticholinergics cause the heart rate to increase. Pulses may be weak, slow, irregular, or even imperceptible.

- Blood pressure requires electronic or mechanical monitors to measure. It will be reduced if blood loss is significant, in shock, or pending cardiac arrest. Cardiac arrhythmias may be noted if electronic monitors are used.

- Capillary refill time progressively slows to 3 or more seconds indicating blood pressure is inadequate to perfuse peripheral tissues (blood loss, shock, pending cardiac arrest).

- Respirations may be slow, irregular, shallow, and often become diaphragmatic, and may eventually cease. Paradoxically respirations may increase in response to low blood O_2 and high blood CO_2 during deep anesthesia. Mucous membrane and skin color (depending on the animal's pigmentation) may be pale to cyanotic from poor perfusion of capillary beds and low blood O_2. Blood loss, decreased blood pressure, shock, and hypothermia reduce blood flow to tissues. Low blood O_2 from hypoventilation causes cyanosis, although tissue perfusion may be normal. Gastrointestinal, ocular, musculoskeletal, and nervous system reflexes may be greatly diminished or cease.

8. **The following corrective actions** should be taken when signs of anesthetic overdose are apparent: turn off or decrease flow of gas anesthetics. If reversible anesthetics are on board, administer a reversal agent. If possible, mechanically ventilate with 100% oxygen. If the animal is not already intubated, insert an endotracheal tube immediately (non-rodents). Administer warm isotonic fluids, intravenously or intraperitoneally (rodents). Administration of fluids to larger mammals is facilitated if an IV line is already in place. Warm the animal to increase body temperature.

G. Analgesics

1. Analgesia must be provided for every animal undergoing a potentially painful procedure including post-operative periods. Analgesics allow a smoother post-operative recovery period. Pain can cause alterations in physiological parameters that may influence research results. <u>The lack of use of analgesics during painful procedures or during the post-operative period must be scientifically justified in writing and approved by the IACUC.</u>

2. **Preemptive analgesia**, managing pain before it begins, holds significant benefits for the animal. If the selected analgesic does not interfere with the research parameters, the data produced can be improved when the stress secondary to pain is removed. Analgesia is always more effective when

given before the painful stimulus is introduced and preemptive analgesia should be used whenever possible, especially for opioids. Analgesics are broadly classified into two groups - the opioids, and non-steroidal anti-inflammatory drugs (NSAIDS). Long acting local anesthetics can also be used for preemptive analgesia.

3. **NSAIDS** (e.g., meloxicam, carprofen, ketoprofen) are effective in ameliorating low to moderate pain. These drugs act by inhibiting the enzymatic production of prostaglandins that are released following tissue damage and affect nociceptors. In addition to providing analgesia, NSAIDS have varying degrees of anti-inflammatory and anti-pyretic activity. Prolonged use of NSAIDS can cause stomach and intestinal ulcers and bleeding as well as nephrotoxicity. NSAIDS are metabolized in the liver and excreted by the kidneys.

4. **Opioids** (e.g., morphine, oxymorphone, butorphanol, buprenorphine, fentanyl, tramadol) act by binding to receptors in the cortex and spinal cord. This group of drugs is most effective at relieving continuous dull pain such as that experienced post-operatively. Opioids also cause hyperthermia, drowsiness, decreased gastrointestinal motility, nausea, vomiting, and alterations of the endocrine and autonomic nervous system. These drugs can produce significant respiratory depression if used incorrectly. The effects of opioids can be reversed or prevented by the administration of naloxone. Fentanyl can be administered as a transdermal patch in large mammals. Opioids are metabolized in the liver and excreted by the kidneys. Note: these drugs are controlled substances and require a DEA license to acquire and use.

5. **Sustained release analgesics** exist as formulations for both NSAIDs (e.g., meloxicam SR) and opioids (e.g. buprenorphine SR and buprenorphine Ethiqa XR). Due to the nature of these products, they cannot be diluted to increase the volume.

6. **Other** non-opioids such as gabapentin or acetaminophen are used for multimodal analgesia or neuropathic pain.

H. Comments Regarding Anesthetics and Analgesics

1. Several commonly used or historically used anesthetics and analgesic medications are described briefly below. However, numerous additional agents are available for use in a variety of species. Contact a CMR Veterinarian for additional information on drugs not listed here. A veterinary drug formulary and a number of veterinary anesthesia textbooks are available for reference.

2. **Acepromazine Maleate** (formerly acetylpromazine), a phenothiazine derivative, is a potent neuroleptic agent with relatively low toxicity. Acepromazine induces tranquilization, muscle relaxation, and a decrease in spontaneous activity. At high doses, sedation occurs. Pre-anesthetic administration decreases the amount of general anesthetic required. Acepromazine possesses antiemetic, anticonvulsant, antispasmodic, hypotensive, and hypothermic properties. Acepromazine will prevent or decrease severity of the malignant hyperthermia syndrome in susceptible swine exposed to halothane. Acepromazine potentiates opiates such as butorphanol and buprenorphine, which if used in combination as a pre-anesthetic, will provide sedation as well as preemptive analgesia. Acepromazine by itself does not provide analgesia.

3. **Fentanyl** is a very potent opiate agonist. (Controlled substance, Schedule II). The patch is a transdermal delivery system for the fentanyl, and is used primarily in large mammals to alleviate

postoperative pain, and to control chronic pain (e.g., associated with cancer). Therapeutic levels are achieved within 6-8 hours of application in the cat, while it takes at least 12 hours to reach therapeutic levels in rabbits, dogs, sheep, and pigs, so patch application should be performed prior to the procedure keeping these times in mind. Animals may be dosed with 2 patches if needed to ensure accurate dosing, but the patch should not be cut in half. Instead, cover ½ of the gel membrane with tape. The hair at the site should be closely clipped with at least a 1-cm margin around the patch. Do not shave, as cuts, abrasions or wounds can alter the absorption of fentanyl. After clipping, wipe the skin with a damp cloth to remove small hairs and skin debris, do not scrub or surgically prepare the site. Allow to completely dry. Place the patch over the clipped area and hold it in place for 2-3 minutes to maximize adherence. Use a slightly padded bandage or transparent dressing used with medical adhesive spray to assure adherence and to keep it dry. Increased temperatures can stimulate an excessive release of fentanyl from the patch, so avoid placing the patch on a location of the animals that contacts the heating pad. Animals should be checked daily to ensure patch is intact and animals should be singly housed while patch is on.

4. **Ketamine** (controlled substance, Schedule III) is a dissociative anesthetic, produces sedation and immobility, increased blood pressure, increased muscle tone, increased salivary secretions, only slight respiratory depression in most species (severe in rodents), variable analgesia, and may cause apnea. In rodents, ketamine must be used in combination with other agents: see tables below.

5. **Local anesthetics**: A variety of local anesthetic agents are available and may be valuable in several types of experimental procedures. For example, local infusion of an incision site with bupivacaine (e.g., Marcaine) and/or lidocaine may reduce the amount of general anesthetic that is required. Application of lidocaine gel to a suture line or a cranial implant or the use of bupivacaine to block intercostal nerves following thoracotomy may provide considerable pain relief.

Use of Bupivacaine in Perioperative Analgesia:

Bupivacaine is a local anesthetic which blocks the generation and conduction of nerve impulses. It is commonly used for analgesia by infiltration of surgical incisions. Preemptive use of analgesics (including local anesthetics used to control post-operative pain) i.e., before tissue injury, is recommended to block central sensitization, thus preventing pain or making pain easier to control.

Bupivacaine has a longer duration of action than lidocaine. Signs of toxicity include central nervous system signs (seizures), and cardiac dysrhythmias progressing to asystole. Bupivacaine toxicity is dose dependent and there is variation between species and age of animals. Rats appear to be more tolerant than larger species (e.g., dogs, sheep), while rabbits are thought to be more sensitive.

Bupivacaine is a prescription drug, but it is not a controlled substance. It is available in concentrations of 0.25% or 0.5%, either plain or combined with epinephrine. Epinephrine reduces cutaneous blood flow and therefore prolongs the local anesthetic effects. Maximum concentration of bupivacaine recommended for subcutaneous use is 0.25%.

Before making the surgical incision, subcutaneously inject small volumes (use a 25-gauge needle) at equidistant places approximately 0.5-1.0 cm apart, in an ellipse around the incision site. Wait 3-5 minutes before starting the incision.

6. **Pentobarbital** (controlled substance, Schedule II) can induce severe cardiovascular and respiratory depression at doses close to those needed to obtain a surgical level of anesthesia and can result in

death. Use of atropine with pentobarbital may lessen these risks. IV administration should be performed slowly and titrated to effect.

7. **Telazol** (controlled substance, Schedule III) is a commercially available preparation of the dissociative tiletamine (50mg/ml) and the benzodiazepine zolazepam (50mg/ml). It is not recommended for use in rabbits (potentially nephrotoxic).

8. **Urethane** use is limited to non-survival procedures.

9. **Volatile anesthetics** include isoflurane. These agents should be used only with an approved gas scavenging system or by using the inhalant anesthetic agent inside an approved fume hood. Precision vaporizers should be used for delivery of these anesthetic agents in survival procedures because lethal concentrations can easily be reached using the open drop method or using a bell jar as an anesthetic chamber.

10. **Xylazine or Dexmedetomidine** are centrally acting alpha-2 adrenergic receptor agonists with analgesic and sedative effects. They can induce profound bradycardia, decreased cardiac output, emesis, and depressed thermoregulation. Ruminants are extremely sensitive to alpha-2 agonists. Yohimbine or atipamezole can be used to reverse the effects of xylazine or dexmedetomidine, respectively.

I. **Summary Tables:** The following tables of drugs are commonly used for pre-anesthesia, anesthesia, analgesia, sedation, tranquilization, and restraint of laboratory animal species. Variations in dose and duration of action will probably be observed due to factors such as animal strain, route of administration, weight, temperament, presence of other drugs, and state of health. Because of these considerations, animal users must be able to judge depth of anesthesia in the individual animal to avoid administration of a lethal dose, or a dose that inadequately controls pain.

Recommendations for types of analgesics for different procedures and expected pain levels

Type of pain	Severity	Examples of procedure	Duration analgesia is provided	Recommended analgesics
Surgical	Mild	Punch biopsy, vascular cutdown	Once	Local +/- NSAID
Surgical	Moderate	subcutaneous procedure	1 full day	Local with either NSAID or Narcotic
Surgical	Moderate-Severe	Craniotomy, catheter implantation	3 full days	Local with either NSAID or Narcotic
Surgical	Severe	Thoracotomy, laparotomy, head cap	3 full days or longer, as needed	Local with both NSAID and Narcotic
Chronic	Mild-moderate	Arthritis	Long term	NSAID

Anesthetics and Analgesics used in Mice

Anesthesia in Mice	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-4% induction, 1-3% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Pentobarbital*	35 mg/kg IV 60 – 80 mg/kg IP	Caution! Potentially significant cardiovascular and respiratory depression, variable response
Tribromoethanol (Avertin)	125-290 mg/kg IP	Store at 4°C; dark conditions. Not preferred anesthetic; needs strong scientific justification
Ketamine* + Xylazine	80-100 mg/kg (K) + 5-12mg/kg (X) IP	If animals appear to be responding to touch, or awakening, re-dose with 25-50% of the initial dose of ketamine
Ketamine* + Xylazine + Acepromazine	80-100 mg/kg (K) + 5-12mg/kg (X) + 1-3 mg/kg (A) IP	
Urethane (ethyl carbamate)	1.3-1.5 g/kg IP	Terminal procedure only. Must justify use of non-pharmaceutical grade agent. Potentially hazardous to workers, must use appropriate engineering controls (i.e. prepared in hood)
Hypothermia (Neonates <4 days old undergoing minor surgical procedures only)	Place for 3 – 4 minutes in ice water or crushed ice	Pup placed in rubber sleeve, submerged to cervical area with resultant 10 minutes of anesthesia
Analgesia in Mice		
Acetaminophen	200-300 mg/kg PO	
Buprenorphine* (Buprenex®)	0.1mg – 0.5 /kg SC	**4-6 hours of analgesia**; do not use with tribromoethanol
Buprenorphine SR* (ZooPharm)	1 mg/kg SC	Up to 48 hours of analgesia
Buprenorphine XR* (Ethiqa)	3.25 mg/kg SC	Up to 72 hours of analgesia
Carprofen (Rimadyl®)	5 mg/kg SC	Up to 12 hours of analgesia
Carprofen (Rimadyl®)	20 mg/kg SC	Up to 24 hours of analgesia
Ketoprofen	20 mg/kg SC	Up to 24 hours of analgesia
Meloxicam	5-10 mg/kg SC	8-12 hours respectively
Meloxicam SR	4 mg/kg SC	Up to 24 hours of analgesia
Bupivacaine	2-2.5 mg/kg SC or intralesional	Do not exceed 8 mg/kg total; use only as preoperative local anesthesia

Note: Mice have a relatively small total muscle mass and are prone to develop muscular atrophy or nerve damage following IM injections. The IM route should be avoided in mice. If drugs must be administered via the IM route, minimal injection volumes (≤0.05 ml), and a 27-30-gauge needle should be used.

* - Controlled substance

Anesthetics and Analgesics used in Rats

Anesthesia in Rats	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-5% induction, 1-3% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Pentobarbital*	30 – 40 mg/kg IV 40 – 60 mg/kg IP	Caution! Potentially significant cardiovascular and respiratory depression, variable response
Ketamine* + Xylazine + Acepromazine	20-50 mg/kg (K) + 2-10 mg/kg (X) + 0.5-1.5 mg/kg (A)	
Ketamine* + Xylazine	40 – 100 mg/kg (K)+ 5 – 10 mg/kg (X) IP	30 – 45-minute duration; may supplement with ketamine only @ 25-50% dose. Reverse xylazine w/Yohimbine (SC or IP), 2mg/kg BW
Methohexital* (Brevital)	40 mg/kg IV or IP (1% solution)	15 - 20 minutes of anesthesia
Urethane (ethyl carbamate)	1.3-1.5 g/kg IP	Terminal procedure only. Must justify use of non-pharmaceutical grade agent. Terminal procedure only. Must justify use of non-pharmaceutical grade agent. Potentially hazardous to workers, must use appropriate engineering controls (i.e., prepared in hood)
Analgesia in Rats		
Acetaminophen	100-300 mg/kg PO	
Buprenorphine SR* (ZooPharm)	1-1.2 mg/kg SC	Up to 48 hours of analgesia
Buprenorphine* (Buprenex®)	0.01 – 0.05 mg/kg SC	**6-8 hours of analgesia; do not use with tribromoethanol**
Buprenorphine XR* (Ethiqa)	0.65 mg/kg SC	Up to 72 hours of analgesia
Ketoprofen	5.0 mg/kg SC	Up to 24 hours of analgesia
Carprofen	5.0 mg/kg SC	Up to 24 hours of analgesia
Meloxicam	1-2 mg/kg SC or PO	Once every 12-24 hours
Meloxicam SR	4 mg/kg SC	Up to 72 hours of analgesia
Bupivacaine	2-2.5 mg/kg SC or intralesional	Do not exceed 8 mg/kg total; use only as preoperative local anesthesia

Note: Rats have a relatively small total muscle mass and are prone to develop muscular atrophy or nerve damage following IM injections. The IM route should be used with caution in rats. If drugs must be administered via the IM route, minimal injection volumes (≤0.3 ml), and a 25-gauge needle or smaller should be used.

*- Controlled Substance

Anesthetics and Analgesics used in Gerbils

Anesthesia in Gerbils	Dose & Route	Comments
Isoflurane (Florane®)	To effect. In general, 3-5% induction, 1-3% maintenance; inhalation.	Precision vaporizer, adequate ventilation or scavenging essential
Ketamine* + xylazine	50 mg/kg + 2 mg/kg IP	20-60 minutes of anesthesia
Ketamine* + dexmedetomidine	75 mg/kg + 0.25 mg/kg IP	
Ketamine* + acepromazine	50-75 mg/kg + 3-5 mg/kg IM or IP	60-90 minutes of anesthesia
Telazol®* (tiletamine+zolazepam)	50-80 mg/kg IM or IP	
Telazol®* (tiletamine+zolazepam) + xylazine	20 mg/kg + 10 mg/kg IP	
Analgesia in Gerbils		
Bupivacaine	2 mg/kg SQ	Use only as preoperative local anesthesia, wait 5 minutes after injection to take effect, do not exceed 2 mg/kg
Buprenorphine*	0.01-0.05 mg/kg SC or IP	6-12 hours of analgesia
BuprenorphineSR*	0.6-1.8 mg/kg SC	48-72 hours of analgesia
Carprofen	5 mg/kg SC or IP	24 hours of analgesia
Dexmedetomidine	50-100 ug/kg IP	Use only for perioperative, sedation with mild to moderate analgesia
Flunixin meglumine	2.5 mg/kg SC	12-24 hours of analgesia
Ketoprofen	5 mg/kg SC or IP	24 hours of analgesia
Meloxicam (Metacam)	2 mg/kg SC or IP	24 hours of analgesia
Morphine*	2-5 mg/kg SC or IP	2-4 hours of analgesia

*- Controlled Substance

Anesthetics and Analgesics used in Hamsters

Anesthesia in Hamsters	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-4% induction, 1-3% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Ketamine*+Xylazine	80 – 100 mg/kg (K) + 7 – 10 mg/kg (X) IP	
Ketamine*+ Dexmedetomidine	70 mg/kg (K) + 1 mg/kg (M) IP	
Analgesia in Hamsters		
Buprenorphine* (Buprenex®)	0.05 – 0.5 mg/kg SC	**4-6 hours of analgesia**
Meloxicam	1-2 mg/kg SC	Up to 24 hours of analgesia
Carprofen	5 mg/kg SC	Between 12-24 hours of analgesia
Ketoprofen	5 mg/kg SC	Up to 24 hours of analgesia
Flunixin meglumine	2.5 mg/kg SC	Between 12-24 hours of analgesia

*- Controlled Substance

Anesthetics and Analgesics used in Guinea Pigs

Anesthesia in Guinea Pigs	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-4% induction, 1-3% maintenance; inhalation	Precision vaporizer required, scavenging required
Ketamine* + Xylazine	40-50 mg/kg (K) + 5 mg/kg (X) IP	Up to 60 minutes of anesthesia
Analgesia in Guinea Pigs		
Carprofen (Rimadyl®)	4 mg/kg SC	12-24 hours of analgesia
Ketoprofen	1 mg/kg SC, IM	Up to 24 hours of analgesia
Buprenorphine* (Buprenex®)	0.05 mg/kg SC	**6 hours of analgesia**
Buprenorphine SR* (ZooPharm)	0.3-0.5 mg/kg SC	12-24 hours of analgesia
Meloxicam	0.2 mg/kg SC	12-24 hours of analgesia
Flunixin meglumine	2.5 mg/kg SC	12-24 hours of analgesia

Note: Guinea pigs often have a large amount of pasty feed in their mouths that can cause airway obstruction when anesthetized. This residue can be removed by gently rinsing the mouth with water before induction of anesthesia. IM injections of ketamine may result in self-mutilation and muscle necrosis. Anticholinergic medication (e.g., atropine @ 0.05 mg/kg SC or glycopyrrolate @ 0.01-0.02 mg/kg SC) may be used to reduce bronchial secretions and salivation. Normal values: body temperature 37.2-39.5°C (99-103.1°F); heart rate 230-380/min; respiration rate 40-100/min.
*- Controlled Substance

Anesthetics and Analgesics used in Rabbits

Anesthesia in Rabbits	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-5% induction, 1-3% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Ketamine* + Xylazine	35 – 50 mg/kg (K) + 5 – 10 mg/kg (X) IM	Minor procedures; up to 45 minutes of anesthesia; can supplement with ketamine @ 25-50% dose
Ketamine* + Dexmedetomidine	25 mg/kg (K) + 0.25 mg/kg (D) IM	
Ketamine* + Acepromazine	25-50 mg/kg (K) + 0.25-1 mg/kg (A) IM or SC	
Propofol	4 - 20 mg/kg IV, slowly to effect	Induction agent; light sedation
Ketamine* + Xylazine + Propofol + Isoflurane	7.5 - 35 mg/kg (K) and 1 - 5 mg/kg (X) IM + 4 - 20 mg/kg IV, slowly to effect + 3-5% for induction, 1-3% for maintenance	For general surgical anesthesia
Analgesia in Rabbits		
Butorphanol* (Torbutrol® 0.5mg/ml)	0.1 – 0.5 mg/kg SC, IM or IV	Up to 4 hours of analgesia
Buprenorphine* (Buprenex®)	0.01-0.05 mg/kg SC	Between 6-12 hours of analgesia
Buprenorphine SR* (ZooPharm)	0.12 mg/kg SC	Up to 72 hours of analgesia
Carprofen	2-4 mg/kg SC or PO	Between 12 hours of analgesia

Ketoprofen	2 mg/kg SC initially, followed with 0.2-0.5 mg/kg daily	Up to 24 hours of analgesia, treatment should not exceed 5 days
Meloxicam	0.5 mg/kg SC	Once every 12 hours
Banamine	1.1 mg/kg IM or SC	Up to 12 hours of analgesia
Fentanyl patch *	Under 4 kg: use 12 mcg/hr >= 4 kg: Use 20 mcg/hr	Should never come in contact with heating source. Should be placed on animal at least 12 hours prior to scheduled procedure.

Note: Anesthetic depth: Adequate anesthesia for surgery can be very difficult to obtain in rabbits, especially when barbiturates are used. Rabbits are prone to develop respiratory depression and edema when anesthetized. Atropinase: Although atropine is frequently administered to anesthetized animals to reduce oral and respiratory secretions and to support heart rate, many rabbits (up to 50%) have circulating atropinase and thus may demonstrate a reduced duration of effectiveness of this drug. Normal values: body temperature 38.5-39.0°C (101.3-102.2°F); heart rate 130-300/min; respiration rate 30-60/min.
*- Controlled Substance

Anesthetics and Analgesics used in Dogs

Anesthesia in Dogs	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-5% induction, 1-4-% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Ketamine* + Diazepam	10 mg/kg (K) + 0.5 mg/kg (D) IV (anesthesia for minor procedures) 5.5 mg/kg (K) + 0.3 mg/kg (D) IV (induction of anesthesia)	Premedicate with an anticholinergic Anesthesia can be maintained with inhalant anesthetic (e.g., isoflurane)
Ketamine* + Midazolam	5-10 mg/kg (K) + 0.1-0.5 mg/kg (M) IV	Minor procedures; premedicate with anticholinergic
Telazol*® (Tiletamine + Zolazepam)	6 – 13 mg/kg IM	Up to 1 hour of anesthesia
Propofol*	4 mg/kg IV to effect (give slowly)	
Analgesia in Dogs		
Acetaminophen	15 mg/kg PO	
Bupivacaine	1-2 mg/kg at surgical site	
Lidocaine	2-5 mg/kg at surgical site	
Buprenorphine SR*	0.03-0.06 mg/kg SC	Between 48-72 hours of analgesia
Meloxicam	0.2 mg/kg loading dose IM, SQ, PO, then 0.1 mg/kg daily	Up to 24 hours of analgesia
Morphine*	0.25 – 2.0 mg/kg IM or SC	2 hours of analgesia
Butorphanol* (Torbutrol® 0.5 mg/ml)	0.01 – 0.02 mg/kg IM or SC	Between 2 – 4 hours of sedation, 1 hr of analgesia
Buprenorphine* (Buprenex®)	0.005 – 0.03 mg/kg SC	Between 3-4 hours of analgesia
Carprofen (Rimadyl ®)	2.2 mg/kg PO or SC BID, or 4.4 mg/kg PO or SC SID,	Between 12-24 hours of analgesia

Fentanyl patch*	<7 kg = 25 mcg patch; 7-20 kg = 50 mcg patch; 20-30 kg= 75 mcg patch; >30 kg= 100 mcg patch	Each dose provides up to 72 hours of analgesia. Should never come in contact with heating source. Should be placed on animal at least 12 hours prior to scheduled procedure.
Sedation in Dogs		
Dexmedetomidine + butorphanol or buprenorphine	5-15 mcg/kg (D) IM + 0.1-0.5 mg/kg (But) IM OR buprenorphine 0.01-0.03 mg/kg IM	
Diazepam	0.2-0.6 mg/kg IV	
Midazolam	0.1-0.5 mg/kg IV	
Acepromazine + Buprenorphine* OR Morphine* OR Hydromorphone* OR Methadone*	0.005-0.03 mg/kg IM or SC 0.01-0.02 mg/kg IM or SC 0.5-2.0 mg/kg IM or SC 0.05-0.2 mg/kg IM or SC 0.2-1.0 mg/kg IM	
Dexmedetomidine + Morphine* OR Hydromorphone* OR Methadone* OR Butorphanol*	5-15 mcg/kg IM or SC 0.1-0.5 mg/kg IM or SC 0.005-0.2 mg/kg IM or SC 0.1-0.5 mg/kg IM 0.1-0.5 mg/kg IM or SC	
Acepromazine	0.05 – 0.1 mg/kg IM, SC	Maximum administer ≤3 mg total

Note: Anticholinergic medication (e.g., atropine @ 0.02-0.04 mg/kg SC, IM, or glycopyrrolate @ 0.02 mg/kg IM, SC) may be helpful in anesthetized dogs to support the heart rate and reduce bronchial secretions, consult a CMR veterinarian. Normal values: body temperature 37.5-39°C (99.5-102.2°F); heart rate 70-120/min, respiratory rate 15-25/min.
*- Controlled Substance

Anesthetics and Analgesics used in Cats

Anesthesia in Cats	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-5% induction, 1-3% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Ketamine* + Diazepam	10 mg/kg (K) + 0.5 mg/kg (D) IV (anesthesia for minor procedures) 5.5 mg/kg (K) + 0.3 mg/kg (D) IV (induction of anesthesia)	Premedicate with an anticholinergic Anesthesia can be maintained with inhalant anesthetic (e.g., isoflurane)
Dexmedetomidine, Ketamine, Butorphanol	0.0325 mg/kg IM (D) + 6.5 mg/kg IM (K) + 0.65 mg/kg IM (B)	Takes effect in 5-10 minutes, can administer another ½ dose if

		necessary. Reverse with 1/3 total volume with atipamezole
Propofol*	4 mg/kg IV to effect (give slowly)	
Analgesia in Cats		
Morphine*	0.05-0.4 mg/kg IM or SC	Up to 4 hours analgesia; caution, mania and excitation with overdose
Robenacoxib (Onsior®)	1 mg/kg PO 2 mg/kg SC	Once every 24 hours for maximum of three days
Meloxicam	0.3 mg/kg PO or SC	Give once only
Buprenorphine* (Buprenex®)	0.005 – 0.01 mg/kg SC or IM	Up to 12 hours analgesia
Fentanyl patch*	<2.5 kg body weight = ½ of 25 µg/hr patch; >2.5 kg bdy wt = 25 µg/hr patch	Place 8 hours prior to anticipated pain; do not apply heat to patch (e.g., from heating pads)

Note: Acetaminophen (Tylenol) may be toxic in cats and should be used with extreme caution in this species. Cats are also sensitive to the toxic effects of aspirin, and fatalities have been reported. Although aspirin can be used in cats, other agents should be considered. Normal values: body temperature 38.0-39.5°C (100.4-103.1°F); heart rate 110-140/min; respiration rate, 20-30/min. Anticholinergic medication (e.g., atropine @ 0.02-0.04 mg/kg SC, IM, or glycopyrrolate @ 0.02 mg/kg IM, SC) may be helpful in anesthetized cats to support the heart rate and reduce bronchial secretions, consult a CMR veterinarian.
*- Controlled Substance

Anesthetics and Analgesics used in Pigs

Anesthesia in Pigs	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-5% induction, 1-4% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Ketamine* + Xylazine	20-33 mg/kg (K) + 2-5 mg/kg (X) IM or SC	Up to 20 minutes of anesthesia; for minor procedures
Ketamine* + Xylazine + Acepromazine	20-33 mg/kg (K) + 2-5 mg/kg (X) + 1-2 mg/kg (A) SC or IM	
Ketamine* + Midazolam*	10-33 mg/kg (K) + 0.5 mg/kg (M) SC or IM	
Ketamine* + Dexmedetomidine + Butorphanol*	4-6 mg/kg (K) + 0.02-0.04 mg/kg (D) + 0.3 mg/kg (B) IM or SC	
Midazolam* + Dexmedetomidine + Butorphanol*	0.15-0.3 mg/kg (M) + 0.02-0.04 mg/kg (D) + 0.3 mg/kg (B)	Reversible with atipamezole 0.1 mg/kg SQ or IM or flumazenil 0.02 mg/kg IV or IM
Midazolam*	0.2-0.5 mg/kg SC or IM	
Dexmedetomidine	0.04-0.08 mg/kg SC or IM	
Telazol*®	4.4-6.6 mg/kg SC or IM	
Ketamine* + Acepromazine	20-33 mg/kg (K) + 1.1 mg/kg (A) IM or SQ	
Ketamine* + Telazol*®	2.2 mg/kg (K) + 4.4 mg/kg (T) IM	Up to 30 minutes of anesthesia
Telazol*® + Xylazine	2.0 - 8.8 mg/kg (T) + 2.2 mg/kg (X) IM	Up to 20 minutes of anesthesia; may produce cardiopulmonary depression

Ketamine* + Telazol*® + Xylazine	2.2 mg/kg (K) + 4.4 mg/kg (T) + 2.2mg/kg (X) IM	Up to 30 minutes of anesthesia; for minor procedures
Ketamine* + Dexmedetomidine	10 mg/kg (K) + 0.1 mg/kg (D) IM	
Analgesia in Pigs		
Aspirin	10 mg/kg PO	Up to 6 hours of analgesia; use enteric-coated tablet
Flunixin Meglumine (Banamine®)	1-4 mg/kg SC or IM	Up to 12 hours of analgesia
Buprenorphine* (Buprenex®)	0.01 - 0.02 mg/kg IM	Up to 12 hours of analgesia
Ketoprofen	1.0 – 3.0 mg/kg SC	Up to 24 hours of analgesia
Carprofen	2-3 mg/kg SC, PO, IM q 12 hours	Up to 12 hours of analgesia
Buprenorphine SR*	0.12-0.2 mg/kg SC	Up to 72 hours of analgesia
Hydromorphone*	0.1-0.2 mg/kg IV, IM, or SC q 2-4 hours	Up to 4 hours of analgesia
Meloxicam	0.1-0.4 mg/kg PO, SC, or IM q 12-24 hours	Between 12-24 hours of analgesia
Fentanyl* Patches	<30 kg use 25 mcg patch; 30-50 kg use 50 mcg patch; >50 kg use 75 mcg patch	Each dose provides up to 72 hours of analgesia; place 12 hours prior to anticipated pain; do not apply heat to patch (e.g., from heating pads). Apply to thin skin behind ears.

Note: Malignant hyperthermia (MH) is commonly reported in swine. The first cardinal clinical sign of MH is an elevation in end-tidal CO_2. MH is characterized by the sudden onset of muscle rigidity, tachypnea, tachycardia, and hyperthermia (rectal temperatures up to 108°F), followed by dyspnea, cardiac arrhythmias, apnea and death. Anesthesia, restraint, stress, and excitement have all been reported to trigger this condition. Anesthetized swine should be monitored closely for the development of hyperthermia. Emergency measures include cessation of the anesthetic, cooling the body with ice water, and the IV administration of sodium bicarbonate and the muscle relaxant dantrolene (2-10 mg/kg). Normal values: temperature 38.0-40.0°C (100.4-104.0°F); heart rate 60-120/min; respiration rate 10-12/min. Anticholinergic: Glycopyrrolate (0.004-0.01 mg/kg IM) or atropine (0.05 mg/kg IM).
*- Controlled Substance

Anesthetics and Analgesics used in Sheep and Goats

Anesthesia in Sheep & Goats	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-5% induction, 1-4% maintenance; inhalation	Precision vaporizer, adequate ventilation or scavenging essential
Diazepam + Ketamine*	0.2 mg/kg (D) + 3 mg/kg (K) IV	Up to 20 minutes of anesthesia; for minor procedures
Xylazine + Ketamine*	0.05 mg/kg (X) + 4-5 mg/kg (K) IV (Goat) 0.2 mg/kg (X) + 4-5 mg/kg (K) IV (Sheep) 0.1 mg/kg (X) + 10-15 mg/kg (K) IM (Goat) 0.2 mg/kg (X) + 10-15 mg/kg (K) IM (Sheep)	Up to 20 minutes of anesthesia; for minor procedures Up to 45 minutes of anesthesia
Telazol*®	2-4 mg/kg IV	Up to 30 minutes of anesthesia
Xylazine + Telazol*®	0.1 mg/kg (X) + 4 mg/kg (T) IM 0.05 mg/kg (X) + 1 mg/kg (T) IV	Up to 60 minutes of anesthesia

Propofol*	2-6 mg/kg IV to effect (give slowly)	Can add 2-6 mg/kg ketamine* IV if propofol alone is inadequate
Analgesia in Sheep & Goats		
Aspirin	50-100 mg/kg PO	Up to 12 hours of analgesia
Flunixin Meglumine (Banamine)	1-2 mg/kg IV, IM	Up to 12 hours of analgesia
Buprenorphine* (Buprenex®)	0.005-0.01 mg/kg IM, SC, IV	Up to 4 hours of analgesia
Buprenorphine SR	0.2mg/kg SC	Up to 72 hours of analgesia
Fentanyl* Patches	2-3 mcg/kg/hr	Place on shaved limb at least 12 hours prior to anticipated pain; do not apply heat to patch (e.g., from heating pads).
Bupivacaine	1-2 mg/kg SC at surgical site	
Sedation in Sheep & Goats		
Acepromazine	0.05-0.1 mg/kg IM, SC	Moderate sedation
Butorphanol + Diazepam*	0.05-0.1mg/kg (B) + 0.05-0.2 mg/kg (D) IV	Up to 4 hours of analgesia
Diazepam*	0.2-1 mg/kg IV, IM	Light sedation
Ketamine*	20 mg/kg IM	Moderate to heavy sedation
Dexmedetomidine*	0.015 mg/kg IM	Moderate sedation
Midazolam* + Ketamine*	0.5 mg/kg (M) + 4 mg/kg (K)	Heavy sedation
Midazolam*	0.3-0.5 mg/kg IM or IV	Light sedation
Xylazine	0.2 mg/kg IV, IM (Sheep) 0.05 mg/kg IV, IM (Goat)	Light to moderate sedation

Note: Medetomidine and Xylazine can produce hypoxia. Goats and sheep may be fasted for 24-36 hours to reduce the possibility of regurgitation and ruminal tympany (bloat). Water may be withheld 6-8 hours. Always intubate with a cuffed endotracheal tube to prevent aspiration if regurgitation occurs. Intraoperatively a stomach tube should always be placed in the rumen to prevent ruminal tympany, especially when positioned in lateral or dorsal recumbency. Normal values: temperature 38.0-40°C (100.4-104.0°F); heart rate 55-120/min (Sheep), 70-130 (Goat); respiration rate 10-30/min. Anticholinergic drugs are not routinely used during ruminant surgery but are beneficial in treating bradycardia: glycopyrrolate (0.022 mg/kg IM, SC) or atropine (0.05 mg/kg IM, SC).
*- Controlled Substance

Anesthetics and Analgesics used in *Macaca* spp

Anesthesia in *Macaca* spp	Dose & Route	Comments
Isoflurane (Forane®)	To effect. In general, 3-5% induction, 1-3% maintenance; inhalation	Precision vaporizer, adequate scavenging essential
Ketamine + Diazepam*	15 mg/kg (K) + 1.0 mg/kg (D) IM	30-40 minutes of anesthesia
Ketamine* + Xylazine	10 mg/kg (K) + 0.25-2.0 mg/kg (X) IM	30-140 minutes of anesthesia; duration is a function of the xylazine dose
Ketamine* + Dexmedetomidine	4-5 mg/kg (K) + 0.015-0.05 mg/kg (D) IM	Up to 60 minutes of anesthesia
Pentobarbital*	20-30 mg/kg IV	30-60 minutes of anesthesia: reduce dose by $^1/_3$ to $^1/_2$ after administration of ketamine
Thiopental*	15-20 mg/kg IV	5-10 minutes of anesthesia

	5-7 mg/kg IV (induction)	After administration of ketamine
Telazol*®	4-6 mg/kg IM	45-60 minutes of anesthesia
Analgesia in *Macaca* spp		
Acetaminophen	10 mg/kg PO	Up to 6 hours of analgesia
Aspirin	20mg/kg PO 125 mg/kg rectal suppository	6 to 8 hours < 24 hours
Ketoprofen	2 mg/kg IM SID to BID	Up to 12 hours of analgesia
Carprofen	2-4 mg/kg PO, SC	Up to 24 hours of analgesia
Ketorolac	15-30 mg/kg IM	
Flunixin Meglumine (Banamine)	2-4 mg/kg, SC	Up to 24 hours of analgesia; only administer postoperatively to conscious animals
Meloxicam	0.2 mg/kg SQ or PO loading dose, then 0.1 mg/kg thereafter	Up to 24 hours of analgesia
Naproxen	10 mg/kg PO	Up to 12 hours of analgesia
Oxymorphone*	0.15 mg/kg SC, IM, IV	Up to 6 hours of analgesia
Meperidine*	2-4 mg/kg IM	Up to 4 hours of analgesia
Morphine*	1-2 mg/kg SC, IM	Up to 4 hours of analgesia
Buprenorphine* (Buprenex®)	0.01-0.03 mg/kg IM	Up to 8 hours of analgesia
Buprenorphine SR*	0.2 mg/kg SC	Up to 72 hours of analgesia
Sedation in *Macaca* spp		
Acepromazine	0.2 mg/kg IM	Moderate sedation
Diazepam*	1.0 mg/kg IM	Light to moderate sedation
Ketamine*	5-20 mg/kg IM	Moderate sedation, immobilization
Xylazine	0.25-0.5 mg/kg IM	Light to moderate sedation

Note: Anticholinergics: Medetomidine and Xylazine can produce bradycardia and hypotension, in particular at the high end of the xylazine dose. These side effects can be prevented by pre-medicating with atropine (0.02-0.05 mg/kg IM) or glycopyrrolate (0.005-0.01 IM). Anticholinergics also reduce bronchial and salivary secretions. Food: Nonhuman primates should be fasted for at least 12 hours prior to elective surgery. Normal Values: temperature 37-39°C (98.6-103.1°F); heart rate 120-180/min; respiration rate 32-50/min.
*- Controlled Substance

Anesthetics and Analgesics used in Zebrafish

Anesthesia in Zebrafish	Dose (All via immersion)	Comments
Tricaine methanesulfonate (MS222)	50 mg/L – sedation; 100-200 mg/L - surgical anesthesia	Only FDA approved anesthetic for use in fish, 21 day withdrawal, buffer with equal weight of sodium bicarbonate
Benzocaine	25-100 mg/L - light anesthesia	Intermittent dosing may be used to maintain anesthesia for up to 7 hours after induction with MS222, buffer with sodium bicarbonate to maintain neutral pH
Lidocaine hydrochloride	300 mg/L - light anesthesia; 325 mg/L - surgical anesthesia	Small margin of safety, also provides perioperative analgesia

Hypothermia	10°C - light anesthesia; 0-4°C - anesthesia	Exposure must not exceed 10 minutes, only useful for minor procedures or transportation in adults, best for larval fish 1-14 days postfertilization
Analgesia in Zebrafish		
Lidocaine hydrochloride	2-5 mg/L for 30 minutes	Lasts up to 90 minutes
Aspirin	2.5 mg/L for 30 minutes	Lasts up to 90 minutes
Butorphanol	0.2-0.5 mg/L	
Buprenorphine	0.005-0.2 ug/mL	
Morphine	48 mg/L	

RUTGERS UNIVERSITY IACUC Document # A2	Pain/Distress Categories **Date Issued:** 05/16/2019 (version 1.0) **Date Revised:** 2/17/2021 (version 2.0)

I. Purpose

The purpose of this document is to provide guidance for investigators in assigning pain and distress categories when preparing IACUC protocols and to ensure consistent recommendations and review of protocols by veterinarians and IACUC members.

II. Introduction

All vertebrate animals used for research or teaching must be assigned to a USDA pain and distress category on the protocol under which they are used. Determination of pain, distress, or discomfort in animals can be subjective. To minimize variability, objective clinical and behavioral scoring systems should be used whenever possible. When this is not possible, PIs must consider that any procedures that would cause pain or distress in humans may cause pain or distress in other animals. Rutgers is committed to enhance animal well-being and eliminate or otherwise minimize any pain and/or distress to mild and momentary levels with available methods. All exceptions to this policy require scientific justification, review and approval by the IACUC. Related regulations, guidelines, & reference resources can be found below. Please note that the following resources may be revised periodically.

- Animal Welfare Act Regulations (9 CFR 2.C.2.36.b.5)
- PHS Policy on Humane Care and Use of Animals
- AVMA Guidelines for the Euthanasia of Animals, current version
- Guide for the Care and Use of Laboratory Animals, current version
- Guide for the Care and Use of Agricultural Animals in Research & Teaching, current version
- AAALAC-approved taxon-specific resources for wildlife and non-traditional species

III. Responsibilities

When preparing an animal care and use protocol, investigators assign each animal or group of animals to one of the four defined pain categories using this policy's guidelines. During protocol review, IACUC members and veterinary reviewers must verify that animals are assigned to the most appropriate category as determined by the expected pain or distress level that is proposed to be experienced by an animal. When an animal experiences unexpected pain and distress that is not already described and justified within the approved protocol, a CMR veterinarian must be consulted promptly.

IV. Definitions

The definitions of "pain" and "distress" as noted in the Guide for the Care and Use of Laboratory Animals (the Guide):

- "Pain is a complex experience that typically results from stimuli that damage or have the potential to damage tissue; such stimuli prompt withdrawal and evasive action" (the Guide, p. 120).

- Stress is a real or perceived perturbation to an organism's physiological homeostasis or psychological well-being. The transition of stress to distress depends on several factors. Of clear importance are stressor duration and intensity, either of which is likely to produce behavioral or physical signs of distress.
- "Distress may be defined as an aversive state in which an animal fails to cope or adjust to various stressors with which it is presented. But distress may not induce an immediate and observable pathologic or behavioral alteration, making it difficult to monitor and evaluate the animal's state when it is present. Both the duration and intensity of the animal's state are important considerations when trying to prioritize attention to and treatment of animal distress." (the Guide, p. 121)
- Humane Endpoint is the point when an animal's pain or distress is ended or reduced by taking action that includes euthanizing the animal, stopping a painful procedure or alleviating the pain or distress with appropriate measures. Body temperature, body weight, tumor ulceration, tumor size, behavioral changes (e.g. reduced exploration, ruffled pelt), pathological changes observed using imaging technology and blood oxygen saturation are examples of criteria that have been used successfully to implement humane endpoints.

V. Pain Categories

Pain categories are defined by the procedures and how the animals experience them; they are not measures of degrees of pain. When animals will be subjected to a series of procedures with different pain or distress levels, the protocol will be assigned the highest level of pain/distress category according to the procedures described. For example, a protocol with oral administration of a drug which would be categorized as a C, and involving subsequent survival surgery with adequate post-operative pain relief will be assigned an overall category D.

Category B

Category B is appropriate for animals that are not undergoing any experimental manipulations.

Examples of category B procedures include:

- Animals being housed without any research manipulation, prior to transfer to another protocol
- Observation of animal behavior in the wild without manipulating the animal or its environment

Category C

Category C is appropriate for animals that are undergoing manipulations that don't involve more than momentary pain or distress and do not require the use of pain-relieving drugs.

Examples of category C procedures include:

- Handling or weighing animals in teaching, outreach, or research activities
- Ear punching of rodents
- Tail snips in mice <21 days old
- Euthanasia of breeding animals or unused offspring (e.g., via CO_2)
- Animals that are euthanized before tissue collection or other manipulations, if no other procedures performed put them in a higher pain/distress category
- Routine procedures such as injections and blood sampling from veins that produce only mild transient pain or discomfort
- Use of anesthesia as a restraint method only to do a procedure that would not cause more than momentary pain or distress in an awake animal

- An observational study of animal behavior where animals do not experience unavoidable pain or distress
- Behavioral tests without aversive stimuli or that do not induce distress
- Feed and water studies which do not result in clinical health problems or weight loss of greater than 20%
- Food restriction in rodents no greater than 24 hours
- Subcutaneously injected tumor cell studies with well described humane endpoints that conform to Rutgers IACUC policies, and are not expected to metastasize internally
- Microchipping via injection (non-surgical)

Category D

Category D is appropriate for animals subjected to potentially painful procedures for which anesthetics or analgesics are used. The important concept is that animals are given pre-emptive or prompt anesthesia, analgesia, or euthanasia at the first observation of pain or distress.

Examples of category D procedures include:

- Administration of drugs, chemicals, toxins, or organisms that would be expected to produce pain or distress, but which will be alleviated by anesthetics, analgesics, or euthanasia agents
- Survival and Non-survival Surgery conducted with appropriate anesthesia and postoperative analgesia. Examples:
    - Removal of a small tumor under local or general anesthesia (classified as a survival surgery)
    - Perfusion (non-survival surgery)
    - Laparoscopy or needle biopsies
    - Tail snips in mice >21 days old
- Retro-orbital injection or blood collection (must be performed under anesthesia)
- Use of analgesia after an animal's skin is exposed to ultraviolet light to cause a sunburn
- Terminal exsanguination (euthanasia by removal of blood) under anesthesia
- Any phenotype that causes pain or distress that will be alleviated

Category E

Category E is appropriate for animals that are subjected to painful or stressful procedures without the use of anesthetics, analgesics, or euthanasia agents. Withholding of anesthetics or analgesics can only be allowed if it is scientifically justified in writing and approved by the IACUC. Non-pharmacological interventions should be considered to minimize pain and distress (e.g., soft bedding, additional enrichment, gentle massage, cold therapy).

Examples of category E procedures include:

- Studies that allow animals to die without intervention (e.g., LD50 studies, mortality as an endpoint)
- Pain studies or other studies that would not be possible if pain-relieving agents are administered
- Psychological conditioning experiments that involve painful stimuli such as a noxious electrical stimulus that cannot immediately be avoided by an animal or a series of noxious stimuli
- Experiments where animals are subject to anxiety and/or depression (e.g., forced swim test without escape)
- Experiments where animals are forced to exercise to the point of exhaustion without an escape
- Experiments where animals develop a painful or distressful clinical disease and will not receive effective treatments (e.g., radiation sickness, microbial virulence testing)

- Studies where animals develop internal tumors (includes surgical implantation, or spontaneous growth and studies where internal metastasis of cutaneous/subcutaneous tumors is expected)
- Exposure to extreme environmental conditions
- Paralysis or immobilization of a conscious animal. Examples:
    - Neuromuscular blocking agents
    - Spinal cord transection
    - Demyelinating disease models
- Any phenotype that causes pain or distress that will not be alleviated

VI. References

- The Guide for the Care and Use of Laboratory Animals, current version
- AVMA Guidelines on Euthanasia, current version
- Animal Welfare Act

RUTGERS UNIVERSITY IACUC Document #B1	Non-USDA Species Survival Surgery
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 1/20/2021 (version 4.0)

I. Purpose

The purpose of this document is to provide investigators with guidelines for conducting acceptable methods of rodent, bird, amphibian, reptile, and fish surgical technique and procedures.

II. Introduction

Rodent surgery must be performed in accordance with the principles set forth in the Guide for the Care and Use of Laboratory Animals (the "Guide"). Rodent surgery can be done following accepted practices of veterinary surgery while making allowances for the unique needs of researchers. Properly performed surgery promotes animal welfare and good science. Surgery performed without proper anesthesia, monitoring and post-operative care can result in poor research data, unnecessary use of animals, animal suffering and suspension of privileges to work on a protocol.

Good surgical technique includes asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and suture patterns.

III. Responsibilities

All individuals performing non-USDA species surgery at Rutgers must comply with the applicable regulations and procedures detailed in this policy. No surgeries can be performed without an Institutional Animal Care and Use Committee (IACUC) approved protocol; this includes practice procedures.

IV. Definitions

Asepsis - The state of being free of living pathogenic microorganisms

Antiseptic – A germicide that is used on skin or living tissue for the purpose of inhibiting or destroying microorganisms

Aseptic surgery - The performance of an operation with sterile gloves, instruments, etc., and utilizing precautions against the introduction of infectious microorganisms from the outside environment

Disinfection – The chemical or physical process of eliminating or reducing harmful microorganisms

Major surgery – Involves invasion of the cranial, abdominal, or thoracic cavities. Any procedure that may leave the rodent with a permanent handicap whether physical or physiological (e.g. limb amputation) or involves extensive tissue dissection or transection is considered major surgery

Minor surgery – Does not expose a body cavity and causes little or no physical impairment (e.g. placement of subcutaneous implants)

Multiple survival surgeries – Involve successive surgical procedures in which an animal is anesthetized for more than one surgical session and/or procedure. Such procedures must be described in the protocol, scientifically justified, and approved by the IACUC.

Sterile - Free from all microorganisms

Sterilization – The process whereby all microorganisms are eliminated or destroyed. The criterion for adequate sterilization is the failure of organisms to grow if a growth-supporting medium is supplied.

Surgery - Any procedure that exposes tissues normally covered by skin or mucosa.

V. Methods

A. Training and Supervision

"Researchers conducting surgical procedures must have appropriate training to ensure that good surgical technique is practiced. The IACUC, together with the AV (Attending Veterinarian) is responsible for determining that personnel performing surgical procedures are appropriately qualified and trained in the procedures." [p. 115-116, *Guide*, 2011]

Persons performing rodent surgery must have appropriate training with supervision. Qualifications are reviewed during the protocol review process. All individuals conducting animal work as part of the research project must be listed in the protocol. Faculty, graduate students, undergraduates, and technicians doing surgery must work under close supervision until the principal investigator or a veterinarian is confident that the surgical and postoperative care is conducted in accordance with generally accepted practices. University veterinarians or other qualified persons may provide training. In house training sessions in basic aseptic and surgical techniques are offered on campuses throughout the university.

The IACUC may require demonstration of surgical competence and compliance with these guidelines on an individual protocol basis. For some protocols, the first surgery after initial approval must be observed by a veterinarian.

B. Elements of Surgery

1. Anesthetics and Analgesics

"An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols." [p. 120, *Guide*, 2011]

The use of anesthetics and analgesics is required and an appropriate method of preventing and/or alleviating pain must be used. Specific choice of agents will depend on the type of procedure, the length of time it will take, the equipment available, and the skill of the individuals performing the procedure. For additional information on anesthesia and analgesia, refer to IACUC Policy Anesthesia and Analgesia in Laboratory Animals. Consult with a CMR veterinarian for agent and dose selection. Agents, routes, and doses must be listed in the protocol and other agents cannot be used without approval. According to the US Government Principles for the Utilization and Care of Animals Used in Testing, Research and Training, unless the contrary has been established, any procedure that is painful to humans should be assumed to be as painful to animals. Pain relief must be provided routinely for potential postoperative pain unless a sound, written, scientific justification is made that pain relief will interfere with experimental results.

Anesthesia Support and Monitoring

Monitor the depth of anesthesia before beginning surgery, and periodically throughout the surgery to ensure the animal remains in a surgical plane of anesthesia. **Anesthetized animals must not be left unattended.**

- Check respiration rate and depth regularly
- Check response to toe or tail pinch
- Color of mucous membranes and loss of reflected eye color (in albino animals)

These provide the surgeon with a good assessment of the animal's status. Increased respiratory rate or positive pinch reflex is an indication that supplemental anesthesia is required. Monitor dosages carefully to avoid overdosing.

All gas anesthesia systems must be appropriately scavenged. If activated charcoal scavenging canisters are used (ex: F-Air canisters), canisters must be weighed and dated before initial use and before and after each use. Canisters should be discarded in accordance with the manufacturer's recommendations, they can be discarded in the regular trash unless otherwise noted. Anesthesia vaporizers must be calibrated at least every two years. For details on monitoring, refer to IACUC Policy Anesthesia and Analgesia in Laboratory Animals.

2. Physical Facilities

"The design of a surgical facility should accommodate the species to be operated on and the complexity of the procedures to be performed. For most survival surgery performed on rodents and other small species such as aquatics and birds, an animal procedure laboratory is recommended; the space should be dedicated to surgery and related activities when used for this purpose and managed to minimize contamination from other activities conducted in the room at other times." [p. 144, *Guide*, 2011]

Physical facilities must be clean and otherwise prepared for aseptic surgery. Surgery must be performed in a room or portion of a room that is easily sanitized and that is not used for any other purpose during the time of surgery. The area to be used must be cleaned and cleared of all extraneous items prior to surgery and no other activities can be conducted in the area during the surgical procedure. Materials stored on open shelving above the surgery area are a likely source of contamination through dust dropping onto the site. Surgery may be performed in an investigator's laboratory, or surgery areas in animal facilities may be utilized. Surgical procedures performed in non-vivarium spaces must be disclosed and approved in an IACUC protocol prior to implementation.

- Clean and disinfect the surface before each surgery

*See IACUC Policy Sanitation and Monitoring of Research Equipment

- Provide adequate lighting
- Provide a warming source (e.g. Thermacage, recirculating warm water blanket, temperature-controlled heating pad (not exceeding species specific normal body temperature), warm water

bottle/glove, hand warmer (for small rodents), or heat lamp) to prevent hypothermia of the animal.

3. Aseptic Surgery Techniques

"Aseptic technique is used to reduce microbial contamination to the lowest possible practical level. No procedure, piece of equipment, or germicide alone can achieve that objective. Aseptic technique requires the input and cooperation of everyone who enters the operating suite. The contribution and importance of each practice varies with the procedure. Regardless of the species, aseptic technique includes preparation of the patient, such as hair or feather removal and disinfection of the operative site; preparation of the surgeon, such as the provision of appropriate surgical attire, face masks, and sterile gloves; sterilization of instruments, supplies and implanted materials; and the use of operative techniques to reduce the likelihood of infection." [p. 118, *Guide*, 2011]

Aseptic surgical technique is an approach to performing surgery with the goal of minimizing the introduction of microorganisms to the surgical site. Performed properly, routine surgery should not require pre- or post-operative antibiotics.

Aseptic technique involves preparation of the facility, the animal, the operator, and instruments and supplies in such a manner that they are sterile to start with and so they can be used in a manner which keeps them sterile.

Tips Only Technique (AKA "No Touch")

"While the species of animal may influence the manner in which principles of aseptic technique are achieved, inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses affecting surgical success, animal well-being, and research results. General principles of aseptic technique should be followed for all survival surgical procedures." [p. 118, *Guide*, 2011]

The Tips Only Technique is a modified approach to rodent surgery that is especially useful for multiple-surgery sessions, but one that may also be used for single surgeries such as stereotaxic procedures. The Tips Only technique assumes that if all of the surgical manipulations are done with the working ends of the instruments, without touching the animal with fingers directly, then it is only necessary that the tips of the instruments be sterile. The Tips Only technique does not strictly meet the *Guide's* requirements for sterile gloves, but it is acceptable when certain guidelines are followed.

Tips Only technique is not acceptable for all animals or procedures. It is not acceptable for rodent species covered under the Animal Welfare Act & Regulations (e.g., hamsters, gerbils). It is not an excuse to circumvent accepted standards of sterile technique. In fact, it requires meticulous attention to detail.

An advantage of the Tips Only technique is that sterile gloves are not required. The surgeon is free to prep animals, introduce a pack of suture material into the sterile field, or make adjustments to a stereotaxic apparatus. The following are guidelines for acceptable use of the Tips Only technique in rodent surgery. Many of these techniques will also be useful in the standard aseptic technique.

a. Declare your intent to use the Tips Only technique in your animal use protocol. The IACUC will determine the suitability of the technique for the proposed procedure.
b. Sterilize all instruments and supplies in advance.
c. Prepare a sterile field on which to place instruments. Establish a line between the area for sterile instrument tips and non-sterile handles. For example, prior to autoclaving, draw a line on a paper drape with a marker. Label each side "sterile" and "non-sterile". Or, create pockets for instrument tips by stapling a folded drape.
d. Handle instruments only by the handles.
e. Do not touch sterile tips with your hands. Do not allow tips to touch non-sterile surfaces.
f. Handle sutures, catheters and other material only with instrument tips.
g. Handle tissues only with instrument tips. Do not touch tissues with your hands.
h. After use, place instruments on the line on the drape with tips on the sterile side.
i. Assign instruments to a particular task. For example, use heavy scissors to cut skin, then use another, finer pair of scissors for cutting internal tissues.
j. Between surgical procedures, clean blood off instruments with sterile saline and a sterile gauze or cotton-tipped applicator. Saline can be kept in a syringe or sterile cup.
k. Use two sets of instruments and alternate sets between animals (optional).
    i. Sterilize instrument tips between surgeries. An effective method to do this is with a glass bead sterilizer. Instruments placed in a cup of hot glass beads are sterile in 15 – 30 seconds. Individual sterilizers vary, so follow the manufacturer's recommendations for use.
    ii. Clean instruments before heating. Allow tips to cool before use.

4. Multiple Surgeries at One Session

One of the greatest challenges in rodent surgery is adapting sterile technique when performing multiple surgeries in one session. If an assistant is available to perform anesthesia, animal prep, and post-op care, the surgeon can use the same gloves and instruments on multiple animals. For simple surgeries, it may be feasible to anesthetize and prep several animals at once. With this technique, a single operator will have to reglove each time sterility is broken. Instruments must be re-sterilized between each animal using a hot bead sterilizer after all blood and tissue is removed from the instruments. This method cannot be applied to all rodent surgeries and is not applicable for USDA-covered species.

5. Instruments

For initial sterilization, steam autoclaving or ethylene oxide gas sterilization is required. Both methods provide dry instruments at the time of surgery. Wrap instruments in such a way that they can be introduced to the surgical field in a sterile manner. Larger surgical packs can be wrapped with fabric or paper wraps. Sterilize smaller packs and individual items in see-through, peel-apart envelopes. Regardless of sterilization and packaging methods, all packs must be marked with an expiration date that is six months from the date of sterilization.

Acceptable methods of instrument sterilization:
- Steam sterilization (autoclave)
- Gas sterilization (ethylene oxide) - requires REHS approval prior to use
- Dry heat (e.g. glass bead sterilizer)
- Gamma irradiation - requires REHS approval prior to use
- Material supplied as sterile by the manufacturer (by any technique) in such a way that it can be introduced to the surgical field in a sterile manner

• Chemical sterilants with adequate contact time (see Table 1 for details)

Unacceptable methods of instrument sterilization:
• Non-sporicidal disinfectants (e.g. alcohol) - see Table 2 "Recommended Instrument Sterilants"

6. Implantable materials

The number and size of implants including but not limited to **cannulae, ports, pumps, biocompatible matrices, etc.)** must be the lowest number and smallest size possible. Implants should not impede normal mobility and physiologic function of the animal (i.e., eating, defecation, urination, or respiration) without scientific justification. The introduction of implants must be described in an approved protocol.

7. Preoperative Concerns

A healthy animal is important to a successful surgical outcome. Rodents with existing clinical or subclinical conditions are more likely to experience complications during anesthesia and recovery. Immunodeficient rodents require special attention to aseptic technique. Since rodents take several days to recover from shipping stress, do not perform surgery for at least 3 days following arrival in the facility. Discuss preoperative fasting with a CMR veterinarian.

8. Animal Preparation

Prepare the surgical site in an area of the lab or surgery room separate from where the surgical procedure will be performed. The animal's skin is a weak link in aseptic technique as the incision site can only be disinfected and not sterilized in the truest definition. Proper preparation will minimize contamination of the surgical field with skin microorganisms.

a. Apply an ophthalmic ointment to the animal's eyes to prevent drying of the cornea.

b. Clip or depilate a large enough area so that hair does not protrude from under the drapes into the surgical field. Remove hair around the surgical site by:
   i. Using a surgical clipper with a #40 blade (clip against the grain) or
   ii. Application of a depilatory cream (follow manufacturer's instructions)

c. Scrub the surgical site with surgical soap. Soap based disinfectant is preferable for initial skin prep as it cleans more effectively. The final prep can be done using a disinfectant solution if preferred. Using a gauze pad or appropriately sized cotton applicator, scrub in a spiral pattern starting over the intended incision site and moving outward. A typical scrub would involve the use an effective disinfectant (See Table 4: Skin Disinfectants) and a rinse with alcohol. At a minimum do at least three cycles of alternating applications of scrub and alcohol. Minimize soaking the body of the animal as this may lead to irreversible hypothermia and death.

d. Sterile Field

   Two steps that improve aseptic technique are a surgical drape on the animal, and the provision of a sterile surface to put the instruments on when not in use. A sterile surgical cover drape is not required by the *Guide,* but is strongly recommended.

Any autoclavable material can be used for draping (e.g., cloth, disposable surgical drape material, paper towels or commercially available pre-sterilized drapes such as Steri-Drapes™). Paper drapes are convenient because they can be customized by cutting a hole using a sterile instrument to fit the surgical site. The larger the sterile field created the easier it is to avoid breaks in sterile technique. Clear plastic drapes are also commercially available and may be preferable to use with small rodents, as they enable the surgeon to visualize the animal during surgery. Another alternative draping material is Glad® Press'n Seal® which is rendered sterile during the manufacturing process. Care must be taken to not contaminate the portions of the drape that will be in direct contact with the animal or instrumentation. Another method of draping is to place the animal in a sterile tubular stockinette.

A sterile surface for placing instruments between use can be: (a) the edge of the sterile animal drape (b) a tray or pan used to sterilize the instruments (c) the inside of the instrument pack wrapper (d) the inside of a glove wrapper or (e) a separate sterile piece of cloth or paper. This subject is discussed in more detail under the Tips Only technique.

9. Surgeon

Minimal Surgeon Apparel

- Clean lab coat or scrub top, remove all jewelry on hands and wrists
- Surgical (i.e., sterile) gloves; scrub hands before putting gloves on. For an exception to this requirement, see "no-touch/tips only technique" above

Supplemental Surgeon Apparel

- Cap, mask, gown
- A sterile gown is recommended for major or prolonged surgeries

10. Operative Period

a. **Hypothermia**

Hypothermia is a common complication of anesthesia and may result in prolonged recovery time from anesthesia or possibly death of the animal. Avoid placing the animal on surfaces that conduct heat (e.g., stainless steel tables, lab benches). Provide a heat source from the preoperative through the postoperative periods. The most optimal thermoregulatory devices are circulating warm water blankets or pads with internal biofeedback. Other suitable devices are warm water bottles, instant heat devices or hand warmers, heat lamps and temperature controlled electric heating pads, but surface temperature should be evaluated prior to use. Cover all devices with a paper towel or other insulation so the animal does not come in direct contact with the device. Body temperature may be monitored during the procedure using a rectal temperature probe. Do not exceed a rectal temperature of 102°F in both rats and mice. During the recovery period, monitor animals to ensure they do not gnaw on or ingest the heating device.

b. **Dehydration**

In prolonged or invasive surgeries, give 0.5-1.0cc (mice) or 2-5cc (rats) of warm sterile saline or Lactated Ringers Solution subcutaneously during and/or after surgery to help prevent dehydration. More may be required if there is extensive bleeding during surgery. Additional

fluids may be given if the animal has not recovered from anesthesia within several hours, though CMR veterinary staff should be contacted for help with prolonged recoveries.

c. **Skin Closure**
There are several choices of methods of skin closure – nonabsorbable or absorbable suture material, wound clips or staples, and medical grade skin glue. Suture sizes for most general purposes for mice are 4-0 or 5-0 and for rats is 3-0 or 4-0. Sutures or staples must be removed from the skin after the incision is healed (generally 10-14 days post-procedure). Do not use braided absorbable suture material for skin closure.

11. Post-Operative Care

> Postoperative care must be consistent with that described in the approved IACUC protocol. All observations, treatments and other care must be documented at the time they are performed. ALL post-operative complications (e.g., wound infections, sick animals, mortality) must be reported to a CMR veterinarian.

Rodents must receive appropriate post-operative care. Post-surgical care includes:

- keeping the animal warm until ambulating
- observing the animal to ensure uneventful recovery from anesthesia and surgery
- administering supportive fluids, analgesics, and other drugs as required
- providing adequate care for surgical incisions; and maintaining appropriate medical records

a. **Move** the animal to a warm, dry area. **Single house animals** on paper towels until fully ambulatory. Do not use bedding that can be inhaled or otherwise obstruct the airway of the animal while it is still anesthetized. Warm the cage to no greater than 30°C (85°F). To prevent hyperthermia, animals must be provided with a means to escape the heat source once they are awake. Provide heat support for half of the recovery cage so the animal can move away from the heat source once ambulatory.

b. **Observe** animals regularly, at least every 15 minutes if using injectable anesthetics, or continuously for inhalation anesthesia until the animal is fully ambulatory. Return the animal to its regular housing only after it has fully recovered from anesthesia.

Administer **analgesics** during surgery or immediately postoperatively. Post-op analgesia must be maintained for at least 72 hours following any major surgical procedure. Minor procedures may only require one dose. Provide analgesics until no signs of pain are present.

c. **Monitor**
After the initial recovery observe animals at least once daily with special attention to the appearance of the surgical site, attitude, food/water intake, elimination, hydration, and weight loss. Attitude and weight loss (or decrease in body condition score (BCS)) are the two most important indicators of health in rodents. Record observations on a daily basis, at the time of observation. Request professional (veterinary) advice if indicated (e.g. animals appear distressed, painful, have decreased body condition/weight, have a decreased appetite or

activity, or any other negative clinical signs). Animals not expected to survive must be euthanized before becoming moribund.

Post-surgical monitoring should include:

i. Observing the animal to ensure uneventful recovery from anesthesia and surgery
ii. Administering supportive fluids, analgesics, and other drugs as required
iii. Providing adequate care for surgical incisions; and maintaining appropriate medical records
iv. Observing incisions for swelling, exudates, pain, or dehiscence (wound rupture or opening)
v. Observing catheters and any other attached devices
vi. Observing for post-procedure related complications such as organ failure, thrombosis, and ischemia

d. **Remove** skin closures (sutures, wound clips) 10-14 days post-operatively.

e. **Surgical Records** must be available for review by veterinary staff and the IACUC. Records for rodent surgery must include enough information to inform the veterinarian/IACUC that surgery has been performed and who to contact should a problem be noted during routine observations.

Post-operative documentation must include the protocol number, procedure type, date and time of surgery, date and time of monitoring, all medications administered (dose and route), general animal appearance (signs of pain, dehydration, food and water intake), and responsible individual with accessible phone. Check with a CMR veterinarian in your facility for the correct procedure for documenting post-operative care.

a. **Antibiotics**: See IACUC Policy Drugs and Materials
Properly performed aseptic surgery should not require the routine use of post-operative **antibiotics**. If post-operative infections become a problem, first evaluate the aseptic technique of the operator. If antibiotics are used prophylactically, start them at the time of surgery and continue use for at least three days. Report all infections to a CMR veterinarian so that specific, individual guidance, may be obtained and antibiotics prescribed, as needed. Please note that routine antibiotics must be on the IACUC-approved protocol.

b. **Assessment of Surgical Outcomes**

"A continuing and thorough assessment of surgical outcomes should be performed to ensure that appropriate procedures are followed and timely corrective changes instituted. Modification of standard techniques might be desirable or even required (for instance, in aquatic or field surgery), but should not compromise the well-being of the animals. In the event of modification, close assessment of outcomes may have to incorporate criteria other than obvious clinical morbidity and mortality. Such assessments rely on continuing communication among technical staff, investigators, veterinarians, and the IACUC" [p. 115, *Guide*, 2011]

When conducted according to these guidelines, rodent surgical procedures should result in a high success rate with few complications. Factors which might increase the incidence of problems include the implementation of new procedures, training of new personnel, and especially difficult surgical procedures. The IACUC and the CMR veterinary staff are obliged to assess the adequacy of current

practices, and to implement changes where necessary. In order to do this the IACUC recommends the use of a Surgical Outcome Log. Maintain a log for each procedure approved for each animal use protocol.

VI. References

- Bradfield, JF, et al., Behavioral and physiological effects of inapparent wound infection in rats. Lab Anim Sci, 42(6): 572-578, 1992.
- Hoogstraten-Miller, S. and Brown, PA Techniques in Aseptic Rodent Surgery. Curr Immunol. 2008 August; Chapter: Unit-1.12-14. Cunliffe-Beamer, TL. Applying principles of aseptic surgery to rodents, AWIC Newsletter, 4(2): 3-6, 1993
- Lovaglio, J and Lawson, PT, A draping method for improving aseptic technique in rodent surgery, Lab Animal 24(10): 28-31, 1995.
- Waynforth, HB and Flecknell, PA, eds. Experimental and Surgical Technique in the Rat. London: Academic Press 1992.
- Perrett-Gentil, MI, Rodent Surgery: Application of Aseptic Technique and Perioperative Care. http://research.utsa.edu/wp-content/uploads/2015/02/Rodent-Surgery-Handout-Application-of-Aseptic-Technique-and-Perioperative-Care.pdf

Table 1. Recommended Instrument Sterilants

Contact times for all agents must be followed as per manufacturers' instructions. Times noted in the table are the minimum requirements.

Agent	Examples	Comments
Physical: Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min vs. 131° C for 3 min)
Dry Heat[1]	Hot Bead Sterilizer Dry Chamber	Fast; Instruments must be cooled before contacting tissue
Ionizing radiation	Gamma Radiation	Requires special equipment
Chemical: Gas Sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissues; all materials require safe airing time. Carcinogenic. Use only for materials which cannot be sterilized with any other method
Hydrogen Peroxide	(Sterad®)	
Chlorine[2]	Chlorine Dioxide (Clidox®, Alcide®), MB10	Presence of organic matter reduces activity. Must be freshly prepared (<14 days)

[1]Instruments must be cleanedbefore being placed in the bead sterilizer.

[2]Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used.

Table 2. Recommended Instrument Disinfectants

Agent	Examples	Comments
Alcohols Primary use is as a disinfectant-soak between animals when starting with sterilized instruments.	70% ethyl alcohol 70%-99% isopropyl alcohol	NOT ADEQUATE FOR PRIMARY INSTRUMENT STERILIZATION. Minimum contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable. Low level disinfectant.
Chlorine[1]	Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®), MB10	Corrosive. Presence of organic matter reduces activity. Chlorine products must be fresh.
Peracetic Acid/ Hydrogen Peroxide	Spor - Klenz®	Corrosive to instrument surfaces. Must be thoroughly rinsed from instruments before use. Highly irritating to the respiratory system. Must be used only in a ducted biological safety cabinet.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

[1]Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used.

Table 3. Skin Antiseptics

Alternating disinfectants is more effective than using a single agent. For instance, an iodophor scrub can be alternated 3 times with an alcohol. Alcohol by itself is not an adequate skin disinfectant. The evaporation of alcohol or alcohol-based products can induce hypothermia in small animals.

Name	Examples	Comments
Alcohols	70% ethyl alcohol 70-99% isopropyl alcohol	NOT ADEQUATE FOR SKIN PREPARATION when used as a sole surgical disinfectant. Not a high-level disinfectant. Not a sterilant. Flammable.
Iodophors e.g. povidone iodine	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbe killing action. Works best at pH 6-7.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.

Table 4. Suture Selection

Suture	Characteristics and common applications
Vicryl®, Dexon®	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable. **Braided, multi-filament sutures are not acceptable for suturing skin.**
PDS® or Maxon®	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.
Prolene®	Nonabsorbable. Inert.
Nylon	Nonabsorbable. Inert. Recommended for skin.
Silk	Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Silk is very easy to use and knot. **Braided, multi-filament sutures are not acceptable for suturing skin.**
Chromic Gut	Absorbable. Versatile material. Causes mild inflammation but is absorbed more rapidly than synthetics. **Chromic gut is not acceptable for suturing skin.**
Stainless Steel: Wound Clips, Staples	Nonabsorbable. Requires instrument for removal from skin.

RUTGERS UNIVERSITY IACUC Document #B2	Non-Rodent Surgery / USDA Species
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 1/20/2021(version 4.0)

I. Purpose

This policy applies to all surgical procedures performed at Rutgers University on species covered under the Animal Welfare Act. It includes animals such as rabbits, cats, dogs, non-human primates, and livestock.

II. Introduction

"An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols." -p. 120, *Guide*, 2011

Properly performed surgery promotes animal welfare and good science. Surgery performed without proper anesthesia, monitoring and post-operative care can result in poor research data, unnecessary use of animals, animal suffering and suspension of privileges to work on a protocol. Good surgical technique includes asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and suture patterns. There are different requirements depending on the species, type of surgery, and activity being performed.

Additionally, the USDA's CFR (Code of Federal Regulations), title 9 (Animal Welfare Act and Regulations) requires that all major operative procedures on non-rodent species are to be performed in a dedicated facility that is operated and maintained under aseptic conditions. It is also required that no animal is subjected to more than one major operative procedure unless the procedure has been justified for scientific reasons by the Principal Investigator and approved by the Institutional Animals Care and Use Committee (IACUC), or the procedure is required as routine veterinary care to protect the health or well-being of the animal.

III. Responsibilities

This document applies to anyone performing surgery on any species covered under the Animal Welfare Act at Rutgers University.

IV. Definitions

Surgery - Any procedure that exposes tissues normally covered by skin or mucosa.

Minor surgical procedure - A surgical operation that does not involve penetrating or opening a body cavity or any surgical procedure which does not produce permanent physical or physiologic impairment (e.g., subcutaneous implant, castration).

Major surgical procedure - A surgical operation that involves penetrating or opening a body cavity or any surgical procedure which produces permanent physical or physiologic impairment, or major dissection/transection of tissues. (e.g., abdominal, thoracic, and some cranial surgeries).

Postoperative period - The period of time after recovery from anesthesia and prior to removal of surgical sutures and or wound healing. Generally, the period will be no less than five days.
Multiple survival surgery – More than one survival surgery is performed on a single animal (animal recovers from anesthesia between procedures); multiple survival surgeries in USDA covered species requires strong scientific justification.

Non-survival surgery (terminal) – Animals do not regain consciousness following the anesthesia and surgical procedures.

V. Methods

A. Training

"Researchers conducting surgical procedures must have appropriate training to ensure that good surgical technique is practiced—that is, asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and pattern.

"The IACUC, together with the AV (Attending Veterinarian), is responsible for determining that personnel performing surgical procedures are appropriately qualified and trained in the procedures."
- p. 115-116, *Guide*, 2011

Personnel involved with anesthesia and surgery in a research setting often have a wide range of educational backgrounds and may require various levels of training before performing surgery on animals. Personnel trained to perform surgery in humans may require additional training for inter-species variation in anatomy, physiology, and response to anesthetics and analgesics.

Regardless of an individual's responsibility or educational background, **all personnel performing anesthesia and surgery must have thorough knowledge and understanding of the approved IACUC protocol procedures and possess knowledge and familiarity with the relevant anatomy of the species and the surgical site.**

At a minimum, training of anesthesia and surgical personnel must include:
- A thorough knowledge of aseptic technique, including sterile gowning techniques
- Administration and assessment of anesthesia
- Appropriate tissue handling (tissue trauma contributes to postoperative infections)
- Familiarity with possible adverse events and when and how to manage properly such events (e.g., cardiac arrhythmias, bradycardia, etc.) in each species used
- Appropriate use of instruments
- Effective methods of hemostasis
- Correct use of sutures and/or skin staples
- Postsurgical care and monitoring, including the ability to recognize and alleviate pain and distress

In order to perform surgery, each researcher must be certified via observation for that procedure by a Comparative Medicine Resources (CMR) veterinarian or veterinarian's designee. Generally, this will require the veterinarian or designee to observe the researcher perform the surgical procedure.

Certification is for an individual researcher performing a specified procedure. New procedures, and/or different species may require separate certification.

Non-certified people may assist and be trained by a certified surgeon. The certified trainer is responsible for the proper performance of the surgery, must be physically present throughout the procedure and must scrub in. An uncertified researcher may not perform surgery alone without being certified by a CMR veterinarian.

CMR veterinarians will provide training in general surgical technique and in specific procedures. Researchers may also obtain training from others, but all surgery done at Rutgers University must be performed by a certified surgeon under an approved animal use protocol.

B. Surgical Facilities

Unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, aseptic surgery on USDA covered species must be conducted in dedicated spaces approved by the IACUC. There is at least one approved space on each of the Busch, Cook, New Brunswick, and Newark campuses. Because these facilities are not always used regularly, the following procedures must be employed:

The surgery suite floor should be mopped with an appropriate disinfectant prior to use. If the surgery suite/room has not been used in the last seven days, mop the floor before the next surgery. This may be done the day before. Clean the surgery suite following the last procedure of the day. (Empty wastebaskets, clean all surfaces, move all equipment and mop floor, clean and store all instruments, etc.). Leave the surgery room in such condition so that it is ready for use by the next researcher.

The operating room must be free of supplies and equipment that are not relevant to the surgical procedures being performed. Long term storage and storage of supplies not used in operative procedures is not permitted.

The number of people present in the operating room must be suited to the size of the room and complexity of the procedure. CMR reserves the right to remove any non-authorized or excessive authorized personnel if their presence interferes with the procedure and/or compromises aseptic technique or the safety of personnel or the research animal.

Preparation of the animal (e.g., anesthetization, clipping and preliminary surgical scrub; see section C.2) must be performed in the animal prep room separate from the operating room. After the animal has been moved to the operating room, perform a final scrub on the operating table.

Preparation of the surgeon must be performed in the surgeon prep room separate from the operating room which must be contiguous with the operating room (see section C.3 for more information). Instrument cleaning and pack preparation may also occur in this area but cannot occur in the operating room.

C. Surgical Preparation

1. Instruments

The use of sterilized instruments is a critical requirement of sterile survival surgery techniques. The preferred methods of sterilization are high pressure/temperature (autoclave) for items that can withstand high temperature, and ethylene oxide gas for items that cannot withstand high

temperature. Sterilization indicators need to be used to identify materials that have undergone proper sterilization.

a. Both methods provide dry instruments at the time of surgery. Wrap instruments so that they can be introduced to the surgical field in a sterile manner. Larger surgical packs can be wrapped with fabric wraps, or paper wraps. Smaller packs and individual items can be sterilized in see-through, peel-apart envelopes. Regardless of sterilization and packaging methods, all packs must be marked with an expiration date that is six months from the date of sterilization.
b. Ethylene oxide requires REHS approval prior to use.

Cold chemical sterilants may be used effectively for some items and must be approved in the IACUC protocol. The use of liquid chemical sterilizing agents must be conducted in approved facilities with adequate ventilation systems and with adequate contact times consistent with the manufacturer's recommendations. Rinse instruments with sterile water or sterile saline before use. Consult REHS prior to use.

Note: **Rutgers does not consider alcohol to be a sterilizing agent.**

2. Animal(s)

The animal's skin is a weak link in aseptic technique as the incision site cannot be sterilized. Proper preparation/disinfection will minimize contamination of the surgical field with skin microorganisms. Hair around the surgical site must be removed (typically with a surgical clipper using a #40 blade). Shave a large enough area so that hair does not protrude from under the drapes into the surgical field.

<u>Animal Preparation - Farm Animal Species</u>

Farm animal species require some special procedures to maintain an aseptic environment. The following procedures should be followed in working with farm animal species:

- Unless contraindicated by the research protocol, shear sheep closely prior to surgery.
- Where possible, large animals (e.g., sheep, goats, swine, etc.) should be brushed or combed to remove bedding, feces, dirt, etc. before being brought to the surgery area. This can be done the day before if animals are placed in a raised floor pen until surgery.
- Clip the surgery site, peripheral vein sites, etc. with a #40 blade. Do this outside the surgery room. This can be done the day before or immediately prior to inducing anesthesia if the animal's temperament permits it, or it can be done in the animal prep room following induction of anesthesia. Do not clip hair in the surgery room.
- Anesthetize animals outside of the surgical area, and carry into surgery or move on a cart.
- Cover the hooves of large animals before the animal is brought into surgery. This can be done with exam gloves, small bags, paper, etc.

Scrub the surgical site with a surgical soap. Scrub in a spiral pattern starting over the intended incision site and moving outward. A typical scrub would involve use of a povidone-iodine soap (e.g. Betadine® scrub) or chlorhexidine scrub followed by a rinse with 70% isopropyl alcohol. At a minimum, do three alternating applications of scrub and alcohol (scrub then alcohol, scrub then alcohol a second time, and scrub then alcohol a third time). It is acceptable to apply a final application of betadine solution (not scrub) after the three alternating rounds of prep.

The surgical site should be covered with a sterile surgical drape. Paper drapes are convenient because you can customize the hole to fit the surgical site. Disposable surgical drape material is resistant to tearing when wet, and the blue/green color helps reduce glare from surgery lights.

Minimal animal preparation:

- Clip hair with an animal clipper using a #40 blade
- Scrub the skin with surgical soap
- Apply sterile surgical drape

3. Surgeon

The surgeon and sterile assistant(s) must scrub their hands and arms with surgical soap and a hand brush for at least 5 minutes before donning the gown and gloves. This requires short sleeves, hence surgical attire (scrubs) is recommended. Do this in an area away from the surgery table (all surgical suites at Rutgers University have a separate room for this purpose).

After using proper hand scrubbing technique, the surgeon steps into the surgical suite to be assisted in putting on a sterile surgical gown and sterile gloves. The surgeon and surgical assistant(s) must wear sterile gowns and sterile gloves for all survival surgery. They cannot touch anything that is not sterile.

All support personnel in the surgery room during surgery must wear a cap, mask, clean or disposable lab coat, and shoe covers, where required. PPE should not be worn outside the surgery suite, and should be removed and disposed as soon as possible after use, especially if soiled. Change shoe covers if worn outside the surgery suite before re-entering.

Minimal Surgeon Preparation:

- Thorough hand scrub with a surgical scrub brush and antiseptic soap
- Sterile gown
- Sterile gloves
- Cap and mask
- Shoe covers, where required

D. Asepsis / Aseptic Technique

Asepsis is defined as preventing exposure to microorganisms and prevention of infection. Three things that are extremely important in achieving asepsis are the reduction of duration, trauma, and contamination.

- **Duration** of surgical procedure is an important factor, as the longer a procedure takes the greater the possibility of contamination and therefore infection.
- **Trauma** that is sustained by the tissue as a result of rough handling, drying out upon exposure to room air, excessive dead space, implants or foreign bodies or non-optimal animal temperatures will contribute to infections.
- **Contamination** refers to the presence of bacteria or foreign matter.

According to the *Guide,* "aseptic technique is used to reduce microbial contamination to the lowest possible practical level. No procedure, piece of equipment, or germicide alone can achieve that

objective. Aseptic technique requires the input and cooperation of everyone who enters the operating suite. The contribution and importance of each practice varies with the procedure."

- p.118, *Guide*, 2011

Regardless of the species, techniques include:

- Preparation of the animal; such as hair removal and disinfection of the operating site(s)
- Preparation of the surgeon such as the provision of decontaminated surgical attire, surgical scrub, and sterile surgical gloves
- Sterilization of instruments, supplies, and implanted materials
- The use of operative techniques to reduce the likelihood of infection
- Antibiotic administration – if warranted

In considering methods of sterilization procedures, it is important to differentiate between sterilization and disinfection. Sterilization kills all viable microorganisms while disinfection only reduces the number of viable microorganisms. High level disinfection will kill most vegetative microorganisms, but will not kill the more resistant bacterial spores. Commonly used surface disinfectants are not acceptable for use in surgical procedures.

E. Anesthesia – The animal must be maintained in a surgical plane of anesthesia for the duration of the procedure. **Anesthetized animals must not be left unattended.**

Methods of anesthesia will vary with the species and procedure(s) being performed and must be detailed in an IACUC approved protocol. Consult CMR veterinarians regarding appropriate choice of anesthetic(s). For further information on anesthetics, refer to the IACUC document "Anesthesia and Analgesia in Laboratory Animals". All gas anesthesia systems must be appropriately scavenged. If activated charcoal scavenging canisters are used (ex: F-Air canisters), canisters must be weighed and dated before initial use and before and after each use. Canisters should be discarded in accordance with the manufacturer's recommendations, and should be discarded in the regular trash or as directed by REHS. CO_2 soda lime must be replaced when there is a uniform purple color. Anesthesia vaporizers must be calibrated at least every two years.

F. Intra-Operative Records

A log must be kept of all surgeries performed under each protocol for each animal (individual animal logs). This log must include the following information:

- Surgical procedure
- Protocol number and PI
- Unique animal number (can be USDA number)
- Date of surgery and start time
- Location and size of IV catheter (if applicable)
- Size of endotracheal tube (if applicable)
- Person performing the surgery (surgeon)
- Fluid type and rate (if applicable)
- Anesthetic dose (must be updated every 15min if using gas anesthesia, as needed for injectables)
- Vital signs such as heart rate, respiratory rate, body temperature, oxygen saturation, end tidal CO_2, blood pressure (updated every 15min)
    - this will vary depending on available equipment, minimally heart rate, respiratory rate, and body temperature must be recorded

- if any of these parameters are changed such that it appears the animal is getting light, all surgical activity must stop, the animal redosed or gas anesthesia increased, and surgical plane must be reestablished before continuing
- Any medications given during the procedure (dose and route)
- Any relevant observations or events during the procedure
- Any complications resulting in anesthetic death
- Time of completion of surgery

Forms may be obtained from the CMR; all surgery logs are kept with the animal's medical records, which remain in the animal facility. Logs must be created in real time and cannot be completed after the procedure. Surgery logs must be available to CMR veterinarians, managers and supervisors, to the IACUC during facility inspections, to USDA, AAALAC or other authorized site visitors, and may be requested as part of periodic review of the protocol approval. Once the animal has been euthanized, the CMR veterinary staff retains all records for an individual protocol for 3 years from the date of termination of the protocol (research staff may retain copies for their records).

G. Analgesia

"An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols." - p. 120, *Guide*, 2011

It is essential that all personnel involved in the care of animals are well-versed in normal animal behavior patterns and even with the individual animal and that they recognize any deviation from the normal or usual pattern. Early recognition of abnormal signs or any deviation from usual daily animal performance can mean the difference between mild, moderate, or severe pain. Review of protocols prior to performance and review of drug literature and analgesics known not to interfere with the experimental design or protocol can enhance treatment of post-procedural pain.

An appropriate method of preventing and/or alleviating pain must be used. Agents should be selected in consultation with CMR veterinarians. Agents, routes, and doses must be listed in the protocol and other agents cannot be used without IACUC approval, unless directed by a CMR veterinarian. A change in the method of analgesia is considered a significant change to the protocol and requires an amendment before the change is made.

For further information on analgesics, refer to the IACUC policy Anesthesia and Analgesia in Laboratory Animals.

H. Post-Operative Monitoring

"Each research facility shall establish and maintain programs of adequate veterinary care that include: adequate pre-procedural and post-procedural care in accordance with current established veterinary medical and nursing procedures." - Animal Welfare Act regulations, sec 2.339b

"During this [post-operative] period, animals should be in a clean, dry, and comfortable area where they can be observed frequently by trained personnel. Particular attention should be given to thermoregulation, cardiovascular and respiratory function, electrolyte and fluid balance, and management of postoperative pain or discomfort. Additional care may be warranted, including long-term administration of parenteral fluids, analgesics, and other drugs, as well as care of surgical incisions. Appropriate medical records should also be maintained.

After recovery from anesthesia, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and to behavioral signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision site for dehiscence, bandaging as appropriate, and timely removal of skin sutures, clips, or staples."
-p.119-120, Guide, 2011

Immediate/acute post-operative period:
Endotracheal tubes must be kept in place until the animal begins to chew or swallow as these are indicators that they are awake enough to protect their airway. An animal with an endotracheal tube in place must not be left unattended.

The animal must be monitored until:

- vital signs are stable
- animal has regained consciousness
- animal can maintain sternal recumbency
- the need for analgesia has been thoroughly assessed
- the animal can easily access and prehend food and water

Maintenance of normal body temperature using blankets and/or other approved medical grade warming sources as well as turning the animal from left to right lateral recumbency every 15 minutes can help decrease recovery times. Food or water should not be available in the cage until the animal is fully recovered or considered stable for the night.

Intermediate post-operative period:
All postoperative animals must be periodically observed, as appropriate for the procedure and species, for the initial 24-72 hour post-surgical procedure period. It is important to assess whether the animal has returned to normal behavior. Animals which do not return to normal behavior often have surgical-related infections/complications and require re-evaluation. Animals must be observed daily until suture removal.

Properly performed aseptic surgery does not generally require the routine use of post-operative antibiotics. If post-operative infections become a problem, the first step should be to evaluate the aseptic technique of the surgeon. If antibiotics are used prophylactically, they should be started at the time of surgery and be continued for at least three days. Clinical infections must be reported to the CMR veterinarians so that specific, individual guidance may be obtained.

The use of post-operative antibiotics, analgesics, or other medications must follow the procedures described in the approved protocol.

Skin closure removal: Sutures / staples must be removed 10-14 days following the procedure unless otherwise directed by a CMR veterinarian.

Post-operative logs: - Typically more detailed logs are maintained for the first 3-5 days post-procedure and then on a daily to weekly basis if no complications arise from the procedure. All logs must be dated (including time) and initialed. Logs will be kept with the medical records in the animal facility until euthanasia; at that time records will be given to the CMR veterinary staff for storage. Copies may be made and retained by the research staff.

VI. Non-Survival Surgery

Asepsis and sterility are not required for non-survival procedures unless the procedures are of sufficient duration to allow bacterial infections to affect the outcome of the study (typically >3hrs). At a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding areas should be clean. Expired materials such as drapes, suture, etc. can be used but note **no expired substances may be administered via any route during surgery**.

VII. References

- Animal Welfare Act: Code of Federal Regulations: 9 CFR Chapter 1 Subchapter A, Parts 1, 2 and 3
- Guide for the Care and Use of Laboratory Animals, current edition

RUTGERS UNIVERSITY IACUC Document #C1	Cancer Studies / Tumor Models
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 2/17/2021 (version 4.0)

I. Purpose

The purpose of this document is to provide information about common tumor models in rodents, monitoring of animals in cancer studies, and limits regarding maximum allowable size of tumors.

II. Introduction

This document applies to the following situations:
1) Tumors induced by injecting cells/tumor fragments into animals (e.g., xenograft or allograft models)
2) Spontaneous, naturally occurring tumors (e.g., geriatric tumors or thymoma in NOD-SCID mice)
3) Induced tumors in mutant mice (e.g., Cre-lox mice, tamoxifen induced models)
4) Chemically induced tumors

Production of monoclonal antibodies using hybridomas in mice is strongly discouraged at Rutgers University and is covered by a separate IACUC document. Investigators must demonstrate that in vitro methods of antibody production or other alternatives cannot be used prior to IACUC approval.

If animals develop spontaneous tumors unrelated to the study objectives, consult a veterinarian for the best course of action – treatment or euthanasia.

III. Responsibilities

This document applies to all investigators performing cancer research (both rodent and human cancer lines) in laboratory animals at Rutgers University.

IV. Methods

A. Pathogen testing of tumor cell lines - Rodent origin cell lines and any biologicals passaged through rodents, including human origin tumors, must test negative for excluded agents as per IACUC Policy Evaluation of Cell Lines and Rodent-Derived Biologicals.

B. Tumor injection sites

- Tumor injection site(s) should be chosen so as not to interfere with normal bodily functions such as walking, eating, drinking, defecation, or urination.
- Whenever possible the tumor should be placed such that it grows with minimal impact on the animal's ability to ambulate and perform normal bodily functions.
- **The recommended site is subcutaneously on the flank, towards the rear of the body**.
- Sites involving sensory functions, such as the eye, should be avoided.
- Intramuscular (IM) implantation should be avoided as growing tumor causes muscle distention and pain.
- Implantation sites on the animal's ventrum should be avoided due to the likelihood of abrasion and ulceration during tumor growth.

- Tumor cell injections are recommended to be performed under anesthesia for safety of personnel.
- Tumor injection site(s) must be disinfected using 70% alcohol before injection.
- **A maximum of two (2) tumor injections are permitted for each animal**.

C. Humane endpoints – or criteria that require euthanasia of animals

1. Tumor size

a. Mice: Single tumors exceeding 2cm in any dimension, or multiple tumors with a combined dimension of ≥2cm
b. Rats: Single tumors exceeding 4cm in any dimension, or multiple tumors with a combined dimension of ≥4cm
c. These measurements are taken from the largest dimension of the tumor
   Example: A mouse has bilateral flank tumors, one is 1 cm in diameter, the other is 1.5 cm in diameter. Combined, they are 2.5 cm, which exceeds tumor guidelines.

2. Tumor volume

a. Spherical tumors: $4/3\ \pi r^3$
b. Ellipsoid tumors: $\pi/6 * (x)*(y)*(z)$

Animals with tumor volumes that exceed 4000 mm^3 in mice or 32000 mm^3 in rats must be euthanized (volume of all tumors on a single animal).

3. Ulcerated/necrotic tumors

In general, the Rutgers IACUC requires immediate euthanasia of any animals with ulcerated tumors and PIs are expected to make every effort to choose models that are not prone to ulceration. Whenever possible, if ulceration is characteristic of the tumor line, the aim of the study should be to complete the experiment in the latent period before ulceration.

If ulcers are justified, researchers must make every effort to analyze data in a timely manner to ensure that experimental endpoints for animals with ulcerated tumors are reached as early as possible. All animals maintained with ulcerated tumors will be considered USDA category E.

Ulceration - Ulceration is typified by necrosis of superficial tissues, due to loss of the epidermis and (at least) the superficial portion of the dermis. Ulcers may be dry (often scabbed over), suppurative (thick, cloudy yellow/green fluid), exudative (thin, clear pink/red fluid), and/or hemorrhagic (bleeding). Any scab on a tumor indicates that ulceration is present. Primary animal welfare concerns for ulcerated tumors includes pain/discomfort, continuous loss of body fluid, and infection.

Tumors that show redness/inflammation on the surface but no open wound or break in the epidermis are not considered ulcerated.

Monitoring:
Pinpoint (≤ 1 mm) ulcerations on any tumor must be monitored at least 3 times per week for worsening of the ulceration site. Observations must be recorded on a tumor observation card.

Ulcerations > 1 mm on any tumor must be monitored daily for worsening of the ulceration site. This includes weekends and holidays. Observations must be recorded on a tumor observation card. These

cards must be kept as part of the research record (scanned and saved digitally or other means) and be available during IACUC inspections for review.

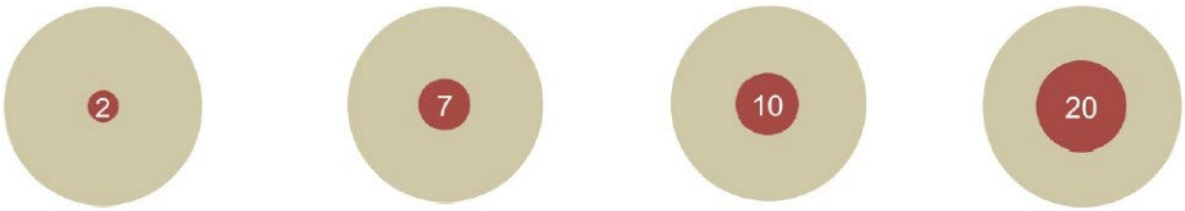
Potential Treatment for Animals with Ulcerated Tumors:

- Animal is bedded on soft substrate such as Alpha-Dri
- Nail trim to avoid irritation from scratching (note: excessive scratching is grounds for euthanasia)
- Application of topical antibiotic/anti-inflammatory cream 1-2 times daily
- Shaving of site (if haired) may help keep area dry and free of debris
- Systemic analgesia (can be supplied via medicated feed or parenterally)

Note: not all of these may be feasible based on the nature of the study

Criteria for Euthanasia:

- Ulcerated tumor(s) actively bleeding which cannot be stopped with gentle pressure applied for 30-60s
- Significant discharge causing dehydration of the animal
- Ulcerated tumor(s) show visible signs of infection including presence of pus and/or foul smell
- Ulceration exceeds 20% of visible tumor surface area:



Note: the number in the center is the percentage of surface area of red circle compared to the beige circle

- Animal shows pain/discomfort associated with tumor ulceration such as biting/scratching at tumor(s)
- Animal shows general signs of pain >24hrs such as hunched posture, piloerection, change in facial expression
- Animal meets other criteria described in Rutgers IACUC Humane Endpoints and Cancer Studies / Tumor Models policies (with the exception of ulcerated tumors) including tumor size
- CMR veterinarians have the authority to require euthanasia for any reason if they feel animal welfare is being compromised based on assessment of animals

4. **Body weight –** Total tumor weight (sum of all tumors on a single animal) cannot exceed 10% of the animal's body weight (measured prior to beginning the study). For immature animals (still growing), expected body weight of an age-matched animal must be used.

Formula to estimate the tumor weight:

$\frac{1}{2}(d^2 \times D) / 1000$ = estimated tumor weight (gm)

'd' and 'D' are the shortest (width) and longest (length) diameters in mm, respectively.

[1000 mg = 1000 mm^3 = 1 cm^3 = 1 cc = 1 gm]

Formula for estimating tumor weight as a percentage of body weight:

(estimated tumor weight (gm) / body weight (gm)) x 100 = tumor weight expressed as percentage of body weight

adjusted body weight = weight of animal with tumor (gm) – estimated tumor weight (gm)

Example:

Estimating tumor weight in a rat using a caliper:
d (width) = 28.3 mm
D (length) = 50 mm
tumor weight = $(28.3mm)^2$ X (50mm/2) = 800.89 x 25 = 20022.25 mm^3 / 1000 = 20 cm^3 = 20 gm

If the rat weighs 220 g with the tumor and the estimated tumor weight is 20 gm, the adjusted rat weight is 200 gm (weight of rat without the tumor).

Using the second formula above: (20/200) x 100 = 10%

The tumor in this animal is 10% of its body weight, which meets the humane endpoint (should be euthanized).

5. **Body Condition Score (BCS)** – Rodents with a BCS < 2 must be euthanized; refer to the IACUC Policy Humane Endpoints document for details regarding body condition scoring.

6. **Tumors that interfere with walking, eating, drinking, urination, or defecation, regardless of size or volume of tumor.**

7. **IACUC Policy Humane Endpoints** – Animals that exceed parameters described in said document must be euthanized.

D. Delivery of tumor fragments/pieces

Tumor fragments are usually delivered subcutaneously through a large-bore needle called a trocar. Because of the large diameter of a trocar, more than momentary pain is associated with their use. Therefore, **all procedures involving trocars are considered minor survival surgery by the IACUC**. Animals must be under general anesthesia and have a local anesthetic agent applied at the trocar site for these procedures. Trocars will cause more damage to the skin compared to smaller gauge hypodermic needles, and skin closure (suture, staples, wound clips, surgical glue) may be needed post-injection. For mice, transplantation of tumor fragments less than 1mm is preferred. Animals should be provided with analgesia for at least 12hrs postoperatively.

E. Animal monitoring in tumor studies

All animals involved in tumor studies must be monitored for pain and distress at least three times per week (at intervals no greater than three days apart) by qualified laboratory personnel. Animals that are approaching humane endpoints (i.e., external tumor diameter ≥1.5cm in mice and/or BCS 2) must be monitored daily, including holidays and weekends.

- Tumor measurement for external tumors: tumor size must be determined at least weekly until tumors reach ≥1.5cm in diameter, at which point tumor size must be determined at least twice weekly or more as needed, based on veterinary recommendation. It is recommended that internal tumor size be monitored via imaging with specific endpoints described in the protocol.
- Body weight measurement: BCS is recommended and must be performed at least weekly. Measuring animal body weight can be misleading as it includes tumor weight.

RUTGERS UNIVERSITY IACUC Document #C2	Demyelinating Rodent Disease Care and Monitoring
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 5/19/2021 (version 3.0)

I. Purpose

The purpose of this policy is to describe the specialized care of rodent models of demyelinating diseases (DMD).

II. Introduction

Although clinical signs vary according to species and strain, DMD typically result in a complex spectrum of acute, chronic, and relapsing-remitting disease that most often result in varying degrees of progressive ascending paralysis.

Due to the extreme variability in the timeline of onset and progression of clinical signs and disease, close monitoring and provision of supportive care are necessary for DMD animals.

III. Responsibilities

This document applies to any animal user using either an induced (e.g., Complete Freund's Adjuvant) or spontaneous DMD model.

IV. Definitions

DMD - demyelinating diseases

BW - body weight

BCS - body condition score

SC - subcutaneous

V. Methods

Care of DMD rodents:

1. Every cage with induced or spontaneous DMD must be identified with a cage card containing the letters "DMD" (can be handwritten on existing cage card) and date of induction, if applicable.
2. Prior to developing clinical signs, spontaneous model DMD animals must be monitored by the lab staff at least twice weekly. Inducible model DMD animals must be monitored by the lab staff at least twice weekly, post-induction.
3. At the first evidence of clinical signs, food and a water source must be easily accessible (e.g., pellets or soft food on floor of cage, long sipper tubes, Hydrogel, and/or gel diets).
4. At the first evidence of clinical signs, the lab staff must monitor animals **at least** once daily, including weekends and holidays, and more frequently as clinical disease progresses. All observations and treatments must be recorded on the post-procedural cage card/log, dated, and initialized.
5. Monitoring should include the graded score of DMD development (see Table 1).

6. DMD rodents must be monitored for skin irritation (dermatitis), urine scalding, penile irritation or prolapse (if male) and tail lesions secondary to flaccid paralysis. Contact CMR veterinary staff for treatment options.
7. Paralyzed animals must be removed and singly housed if they are in a cage with healthy rodents. Healthy animals may walk on paralyzed animals, causing discomfort and/or injuries, and may eat food intended for paralyzed animals.

Table 1 - DMD Clinical Signs and Care

Stage / Grade	Clinical signs	Required Actions
0	**Normal,** no overt signs of disease	1. Record baseline weight 2. Write "DMD" on record cage card 3. For induced models, record induction date on cage card
1	**Tail tone:** Decreased tail tone	1. Start record keeping (activity, DMD stage #) 2. Monitor daily
2	**Hind limb Paresis:** Weakness in hind limbs, animals have difficulty moving, appear ataxic or "clumsy"	1. Monitor and record BW and/or BCS 1X a week 2. Use pellets or soft food on floor of cage, long sipper tubes, Hydrogel, and/or gel diets
3	**Hind limb paralysis:** Inability to move one or both hind limbs **Urinary Incontinence:** Urine is leaking, urine scald around prepuce or vulva **Dehydration:** Decreased skin turgor **Oral/lingual paralysis:** Inability to swallow	In addition to required actions above: 1. Palpate bladder, express at least twice daily if atonic (unable to urinate) 2. Monitor for skin lesions, urine scald, penile prolapse 3. Give daily SC fluid for dehydration, if needed 4. Use softer bedding materials (such as ALPHA-dri®), if animals develop signs of skin irritation 5. Provide food via oral gavage if animal unable to swallow
4	**Weakness of fore limbs with paraparesis or quadriparesis** **Atonic bladder:** Enlarged, unable to urinate **Dehydration:** Decreased skin turgor **Oral/Lingual paralysis:** Inability to swallow	Animals must be euthanized unless scientifically justified in the approved IACUC protocol and CMR veterinarians must be consulted for monitoring and care
5	**Quadriplegia:** Paralysis of all four limbs **Atonic bladder:** Enlarged, unable to urinate **Dehydration:** Decreased skin turgor **Oral/lingual paralysis:** Inability to swallow **Dyspnea:** Difficulty or abnormal breathing **Moribund:** Minimally responsive, hypothermic +/- abnormal breathing	Animals must be euthanized immediately

Humane endpoints (euthanasia indicated):

- DMD grade 4 (unless scientifically justified in the approved IACUC protocol)
- DMD grade 5
- Body Condition Score (BCS) <2 or prolonged body weight loss over 30%
- CMR veterinarian directive

This table should be posted in animal rooms containing DMD rodent cages.

RUTGERS UNIVERSITY IACUC Document #C3	Field Studies
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 6/16/2021 (version 3.0)

I. Purpose

The purpose of this document is to ensure that field studies involving animals at Rutgers University or conducted by Rutgers University personnel are performed according to state, federal, and international laws and regulations and to ensure the proper care and use of animals in field research.

II. Introduction

Field studies are subject to a wide body of regulations on the state, federal, and international levels. Where the AWA and the PHS Policy apply, an IACUC protocol is required, unless **all** of the following conditions are met:

- Experiments/observations do not involve invasive procedures or manipulation of animals
- No harm is done to any animal
- Experiments/observations do not involve alteration of the behavior of an animal during field research activities. For example, any action that is designed to manipulate or alter the behavior of an animal (e.g., making noise to see behavior change) would not meet this condition and therefore not be exempt.

However even if a study is exempt by this policy, PIs should check with the funding source and/or prospective journals to determine if an IACUC-approved protocol is required.

For studies requiring invasive procedures or manipulation, procedures that may potentially harm animals, or studies that involve the intentional material alteration of animal behavior, the Animal Welfare Act and Regulations (AWAR) §2.38 (f)1 apply and: "Handling of all animals shall be done as expeditiously and carefully as possible in a manner that does not cause trauma, overheating, excessive cooling, behavioral stress, physical harm, or unnecessary discomfort." Such studies involving vertebrate animals require IACUC review and approval prior to onset of research.

The IACUC must also review risks to human health that are posed by field studies. The Guide for the Care and Use of Laboratory Animals (the Guide) states that field studies which may involve "Occupational health and safety issues, including zoonoses, should be reviewed by the institution's health and safety committee or office, with assurances to the IACUC that the field study does not compromise the health and safety of animals or persons in the field. Additionally, the investigators conducting field studies with animals should assure the IACUC that collection of specimens or invasive procedures will comply with state and federal regulations."

Foreign countries may have specific regulation(s) or policies that impact international field studies involving methodology, personnel, transportation, shipment of specimens, and permitting. Additional time may be required to obtain the necessary permits and licenses and the IACUC may request to review these documents prior to granting final protocol approval.

III. Responsibilities

This policy applies to all animal users conducting field studies.

Principal Investigator (PI) – PIs must be knowledgeable about animal welfare, relevant zoonotic diseases and occupational health concerns, associated safety issues, and any laws and regulations that apply. It is the responsibility of the PI to understand them, and to **obtain any necessary international or domestic permits/licenses (state and federal) prior to the onset of research and maintain current permits throughout the completion of the study**. The PI must be appropriately qualified and experienced in conducting procedures on living animals and is ultimately responsible for their studies involving wild animals and field studies.

IACUC - In addition to review and approval of those field study protocols that are not exempt from the regulations, the IACUC must also review the likelihood of disease spread from the study animals (zoonoses), and occupational health and safety issues, so that the field studies do not compromise the health and safety of other animals or of persons working in the field.

Institutional Biosafety Committee (IBC) – IBC review and approval may be required for field research with animals and animal tissues that pose zoonotic disease risk.

The federal regulation AWAR §2.31(c) (2) states that "Animal areas containing free-living wild animals in their natural habitat need not be included in such (semiannual) inspection." Per OLAW guidance, the IACUC may, in its role to "consider risks to personnel, and impact on study subjects" request "written descriptions, photographs, or videos that document specified aspects of the study site." The semi-annual inspection process includes submission of a self-assessment questionnaire to the IACUC Office. Additionally, the IACUC may request recordings or other pertinent documentation of the site. Any recordings must follow the IACUC Policy Photography of Laboratory Animals at Rutgers.

IV. Definitions

Field Study - Any study involving free-ranging wild animals (including on any Rutgers campus) or any study involving animals not housed in the University facilities that involve invasive procedures, and/or manipulations in which the animal's behavior is materially altered. This includes wild caught animals that are brought to Rutgers for further use. Procedures may include, but are not limited to, surgery (e.g., transmitter implant), darting, anesthesia, tranquilization or sedation, trapping for placement of identification tags or sample collection (e.g., blood, hair, feces) from any animal, any confinement, transportation, euthanasia including anytime there may be a potential for zoonotic concerns.

V. Methods

A. The information listed below must be considered and included in the protocol for field studies.

1. Species Selection: the investigator should provide information on the species and population(s) to be studied, rationale for such choice(s), and risk to those animals.

2. Site Selection: The investigator should explain how the chosen study location is appropriate for data collection. The investigator must explain procedures and standards used at study sites to reduce potential disruption to the animals and their environment.

3. Methodology Employed: The potential short- and long-term effects of procedures on individual animals, as well as species-specific information, should be described. PIs should consult the relevant taxonomy guide for field studies, where available (e.g., Guidelines for Mammalogy, Guidelines for the Use of Fish in Research, etc.).

   a. If animals are to be captured or trapped, describe the method used, how long they will be kept in a contained environment, and what will be done to reduce the potential for pain and distress. Appropriate provisions for access to food and water must be described.

   b. If animals are to be monitored individually, describe whether they will be identified by natural or artificial markings. If animals are marked by investigators, the process of marking must be described.

   c. Describe the possible impact of capture on subsequent behavior and survival of animals.

   d. Describe procedures to be performed following capture (e.g., blood collection, anesthesia, euthanasia). Describe the degree of invasiveness of the procedure and, where relevant, potential problems associated with the animals' return to the field (e.g., avoiding predators, seeking shelter, surviving inclement weather).

   e. Describe measures taken to prevent injuries and reduce the potential for pain and distress during capture, trapping, marking, or sampling. **Contingency plans for potential adverse events that may occur while the animals are in captivity must be described.**

   f. Consideration should be given to drug availability at the field location and if there are potential barriers to import, transport or storage.

   g. The investigator should consult the most current version of the AVMA Guidelines for the Euthanasia of Animals, which includes considerations and techniques for euthanasia of wildlife.

   h. Describe and justify procedures involving site manipulation. Such manipulations can include transportation of animals, plants or other organisms, physical alterations of the natural habitat, the placement of artificial barriers such as walls or nets or the placement of signal-generating devices.

   i. If animals are to be transported during the study, describe the precautions that will be taken to secure the animals, maintain species appropriate temperature and space, and strategies to reduce stress.

   j. **Describe the precautions taken by the researchers working with animals to protect themselves from possible zoonotic diseases or injury.**

   k. Describe any procedures that have the potential to cause pain and or distress in the animals. These outcomes must be described along with appropriate scientific justification.

   l. The investigator must assure that all personnel handling animals while working in the field are properly trained and knowledgeable of the procedures.

VI. Permitting Agencies and Regulations (including but not limited to)

A. Fish and Wildlife Services: Permits are issued by the U.S. Fish and Wildlife Service (USFWS) under federal regulations 50 CFR 1-100 specifically 50 CFR 13.

B. CITES (Convention on International Trade in Endangered Species): International treaty codified in U.S. law as part of the Endangered Species Act. It regulates import and export of wildlife and plants listed on its three appendices. For more information, go to http://www.cites.org/.

C. Endangered Species Act: Prohibits the taking of any species listed as endangered or threatened. The endangered species list is found in 50 CFR 17.11. Exceptions are made for scientific research and for activities that will enhance the survival of the species. Permits are required for such activities and are issued by USFWS.

a. For more information, go to http://www.fws.gov/laws/lawsdigest/esact.html

D. Drug Enforcement Administration (DEA): Pertains to research involving the use of controlled substances.

E. Lacey Act: Not specific to research but pertains to research involving the import and export of wildlife (50 CFR 14). While the regulations require import of wildlife through designated sites, for scientific purposes wildlife can come through non-designated ports.

F. Marine Mammal Protection Act (MMPA). The 1988 amendments include the listing of conditions under which permits may be issued to take marine mammals for the protection and welfare of the animals, including importation, public display, scientific research, and enhancing the survival or recovery of a species. Scientific permits are provided for by 50 CFR 18. For further information go to http://www.nmfs.noaa.gov/pr/laws/mmpa/

G. Migratory Bird Treaty Act (MBTA): The specific provisions of the statute are described under 16 U.S.C. 703. The title MBTA is a misnomer because the Act does not apply only to birds that migrate long distances or across international borders, but to nearly 830 species of birds. Permits for MBTA are found at 50 CFR 21. Branding and marking activities require a permit under 50 CFR 21.22. These permits are issued by the U.S. Geological Survey-Biological Resources Division's Bird Banding Laboratory. Other permits for scientific collecting (50 CFR 21.23) are obtained from USFWS. For further information go to http://www.fws.gov/birds/policies-and-regulations/laws-legislations/migratory-bird-treaty-act.php

H. Wild Bird Conservation Act (WBCA): Prohibits the import of any bird into the United States other than those specifically listed in the regulations as permissible. For any other species a permit is required. Permits may be issued for scientific research. This law supplements CITES. The regulation for scientific permits is found at 50 CFR 15.22. For more information go to http://www.fws.gov/international/laws-treaties-agreements/us-conservation-laws/wild-bird-conservation-act.html

I. Required Site-Specific Permits

These permits are in addition to the permits described in section C. A permit to conduct research on federal property confers no right to conduct research without other legal required permits.

1. **Bureau of Land Management** (BLM): To determine if any special permissions may be required, contact the nearest BLM Field Office to discuss your proposed research.

2. **National Park Service (NPS)**: The NPS policy for research pertains to all scientific research, application procedures and requirements for research and collecting permits, and the Guidelines for Research and Guidelines for Study Proposals. Researchers are required to submit research proposals, which are reviewed by the NPS for scientific validity and actual or potential impact on park resources, among other things. A specimen collection permit may be issued only to an official representative or a reputable scientific or educational institution or a State or Federal agency for specific purposes described in the regulations (36 CFR 2.5).

3. **National Forests**: Forest Service laws and regulations prohibit all activities that are not expressly allowed by regulations or permit under 36 CFR 251 and 36 CFR 251.54. These regulations do not specifically address scientific research.

4. **State Laws and Regulations:** Virtually all states regulate activities involving wildlife, including scientific research. Please consult the handbook entitled *"Wildlife Laws Handbook"* for further information. Most state regulations also require permits for research on state-owned lands.

VII. References

- Animal and Plant Health Inspection Service, USDA. US Animal Welfare Act (AWA 1990) and Regulations (PL-89-544; USDA 1985) 2005. CFR Title 9, Chapter 1, Subchapter A, Part 3, Subpart D, 3.81d - Animal Welfare. U.S. Government Printing Office, Washington, D.C.
- Public Health Service. 2002. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Publication of the Department of Health and Human Services, National Institutes of Health, Office of Laboratory Animal Welfare.
- Guide for the Care and Use of Laboratory Animals, current edition
- OLAW FAQ Program Review and Inspection of Facilities: Is the IACUC required to inspect field study sites?
- AVMA Guidelines for the Euthanasia of Animals (current version)

RUTGERS UNIVERSITY IACUC Document #C4	Irradiation of Rodents
	Date Issued: 6/17/2020 (version 1.0) **Date Revised:** 5/19/2021 (version 2.0)

I. Purpose

This policy describes common procedures, care, and monitoring for irradiation of rodents.

II. Introduction

Irradiation preferentially kills rapidly dividing cells, including bone marrow and epithelial cells of the gut and other organs, through the introduction of random breaks in DNA. The degree of cellular damage depends on the dose of radiation, age, and strain of the rodents (along with experimental conditions). The most common application of rodent irradiation is to destroy the bone marrow and other hematopoietic progenitors, either for immunosuppression or to replace the immune system of the test subject with donor grafts (bone marrow transplant).

III. Responsibilities

Any animal user irradiating rodents at Rutgers University must comply with this document.

IV. Definitions

Irradiation in this policy refers to the use of ionizing radiation on rodents. Sources are typically gamma rays or medium to high-energy X-rays.

Gray (Gy) is the SI unit of absorbed dose.

Rad is an antiquated term to describe a unit of absorbed dose; however, it is still commonly used in the USA. 100 Rads = 1 Gray.

Fractionation of dose - The total irradiation dose can be split into two or more equal parts separated by a time interval (usually 2-12hrs) in order to minimize morbidity and mortality.

V. Methods

A. General comments about rodent irradiation:

- Fractionated doses should be strongly considered, if appropriate, to reduce morbidity and mortality.
- A pilot study to determine the best dose is recommended if the PI is starting a new study or using a new strain of mice.
- A CMR veterinarian or supervisor should be notified when immunocompromised mice are being subjected to irradiation for proper monitoring and care.

B. Non-Myeloablative Irradiation (NMI) in Mice

- NMI can render an animal transiently immunosuppressed; this is useful for engrafting some tumors and hematopoietic stem cells. NMI is often used in conjunction with immune-deficient rodents to

inhibit innate immune rejection, particularly by natural killer cells, or to enable the development of chimeric animals expressing several types of hematopoietic cells.

- NMI is also used to induce cellular damage in the test subject, often in conjunction with long term cancer studies as NMI may lead to future tumor formation, especially in susceptible strains.
- Depending on the dose, strain, and other experimental conditions, mice experiencing NMI may require some or all of the supportive measures described in section C (Myeloablative Irradiation (MI) /Bone Marrow Transplantation (BMT) in Mice). This determination may be made in part with a Rutgers veterinarian.

C. Myeloablative Irradiation (MI) / Bone Marrow Transplantation (BMT) in Mice

Requirements for MI/BMT studies in mice:

- Animals must be monitored at least daily by the lab staff and findings documented on post-procedural cards for the first 14 days post-irradiation. However, after protocols have been established and PI has experience with the particular strain of mice and dose, three times per week monitoring (with documentation) is acceptable based on CMR veterinary discretion and IACUC protocol details. If animals experience significant (as determined by a CMR veterinarian) morbidity or mortality, daily checks must resume.
- The IACUC Policy Humane Endpoints must be followed. However, body weight loss up to 25% is acceptable during the first 2 weeks post-irradiation for MI/BMT. Experimental and humane end-points must be specified within the IACUC protocol.
- The MI/BMT procedure is automatically classified as category "E" (stress category).

Effects of MI:

- In general, C57Bl/6 mice are more radio-resistant than BALB/c mice. PIs are expected to review literature regarding strain vulnerability to radiation prior to beginning the work.
- Animals may experience irradiation-related sickness generally up to 14 days post-irradiation and generally recover within 2-3 weeks. CMR recommends that animals not be included in experiments during the recovery period.
- Clinical presentation of irradiation includes:
    1. Mice may appear lethargic with a rough coat and assume a hunched posture due to radiation-induced tissue damage and inflammatory responses.
    2. 1 – 3 days post irradiation mice can become dehydrated due to decreased water consumption and develop diarrhea from radiation-induced damage to the intestinal epithelium. Dehydration must be treated as needed by SC administration of any sterile isotonic saline.
    3. Body weight loss up to 25% (due to inappetence and diarrhea) peaks at about 7 days post-irradiation. Depending on the dose and whether immune reconstitution had been provided, recovery will usually occur in 2 to 3 weeks. Mice may never regain their original, pre-irradiation body weight.
    4. Dark stool (melena) or blood-stained perianal area may be present due to intestinal bleeding. Animals may appear pale, especially around the nose and paws due to anemia.
- Severe bacteremia/septicemia may occur. This may require the use of antibiotics, typically administered through the water or feed.
- Successful survival of a bone marrow graft requires suppression of the host's immune system. If the irradiation dose is too low, Graft Versus Host Disease (GVHD) will ensue. As in humans, older mice are more susceptible to develop GVHD.
- Black mice, such as C57Bl/6, will frequently turn gray after irradiation.
- The development of neoplasia after irradiation has been reported in humans and many large animal species. This may occur in mice on long-term studies as well.

- One non-neoplastic illness reported in mice is incisor damage and subsequent difficulty in eating. Providing softened food during the recovery phase is required; teeth trimming may be required.

Care of MI/BMT mice:

- **PIs (or designated research staff) must check the animals daily** for the first 14 days or 3 times per week, based on CMR veterinary discretion and IACUC protocol details. Their condition and care must be documented on the rodent post-procedure monitoring card. BCS or body weight measurement should be performed and recorded on the health monitoring cards until mice return to normal condition, usually within 2-3 weeks. Vivarium staff may provide special care in an emergency and in such a case the PI may be charged accordingly.
- **Use of antibiotics in the drinking water or feed** - Administration of antibiotics in the drinking water or feed may minimize bacteremia/septicemia. PIs are responsible for placing rodents on antibiotic-medicated water or feed at least 1-2 days before the scheduled irradiation in order for the animals to acclimate to the taste. Rodents are typically maintained on antibiotic-medicated water or feed for at least 14 days and up to 28 days post-irradiation. Use of antibiotics must be specified in the approved animal use protocol. Water should be autoclaved, although in some cases reverse-osmosis water may be acceptable. An example of an appropriate antibiotic-medicated diet is LabDiet® PicoLab® 5TK4 (0.124% sulfamethoxazole and 0.025% Trimethoprim).
- **Make drinking water and food readily available** - Irradiated mice will likely experience radiation sickness and not feel well for the first 7-14 days. It is important to provide easy access to sterile water.
- **Gel diet or gel water** is strongly recommended to be provided on the bottom of the cage during the first 14 days following irradiation. ClearH_2O is a commercial vendor that sells these products and includes DietGel (dietary replacement gel) and HydroGel (water replacement gel). **Gel diet/water becomes contaminated with fecal material quickly and must be replaced frequently, typically every 2-3 days.** The animal facility supervisor should be contacted in advance of the planned irradiation event to request diet and water gel be made available in the animal colony room. The outside surface of the gel cups must be sprayed with a suitable disinfectant (e.g., chlorine dioxide product) prior to placement within the cage.
- **Housing** - It is important to realize that after bone marrow transplantation, lethally irradiated mice are severely immunosuppressed for the first two weeks and providing a completely sterile environment (cage, food, and water) is recommended and is required if post-irradiation complications occur.

Recommended antibiotic drugs and preparation (for water bottles) for MI/BMT mice, assuming 25g body weight and daily water consumption of 6.5ml:

drug	stock conc	dose	preparation	frequency	duration	final conc in bottle
Ampicillin	50mg/ml	134mg/kg/day	2.6ml stock +250ml H_2O	Change bottle every 3 days	14-28 days	0.52mg/ml
Baytril	22.7mg/ml	40mg/kg/day	1.7ml stock + 250ml H_2O	Change bottle every 3 days	14-28 days	0.15mg/ml
Sulfamethoxazole-Trimethoprim (SMX-TMP)	Sulfatrim = 200mg SMX + 40mg TMP per 5ml	95mg/kg/day	3.3ml stock + 250ml H_2O	Change bottle every 3 days, keep protected from light	14-28 days	0.52mg SMX, 0.10mg TMP per ml

D. Irradiation of Rats

- In general, the basic principles of NMI and MI in mice outlined in this policy apply to rats, especially in regard to supportive care. Significantly less specific data is available regarding NMI and MI/BMT in rats.
- Pilot studies may be necessary prior to initiating a full study.
- Non-Myeloablative Irradiation (NMI) in rats: Rat NMI is most frequently used as a model for radiation-induced brain injury (Yang 2017). In the Yang study, the radiation dose ranged from 0.5Gy to 40Gy.
- Myeloablative Irradiation (MI) / Bone Marrow Transplantation (BMT) in rats: One source reports 14.4 Gy (whole body) as myeloablative in Wistar rats (Kassayova 1999) while another lists 10Gy (Wistar, whole body, Mihandoost 2014). A recent study in rats includes 7.5Gy as myeloablative in Sprague-Dawley rats (Zawaski 2017).

Recommended antibiotic drugs and preparation (for water bottles) for MI/BMT rats, assuming 300g body weight and daily water consumption of 27ml:

drug	stock conc	dose	preparation	frequency	duration	final conc in bottle
Ampicillin	50mg/ml	134mg/kg/day	15ml stock +485ml H_2O	Change bottle every 3 days	14-28 days	1.5mg/ml
Baytril	22.7mg/ml	40mg/kg/day	30ml stock + 470ml H_2O	Change bottle every 3 days	14-28 days	1.33mg/ml
Sulfamethoxazole-Trimethoprim (SMX-TMP)	Sulfatrim = 200mg SMX + 40mg TMP per 5ml	53mg/kg/day	5.5ml stock + 500ml H_2O	Change bottle every 3 days, keep protected from light	14-28 days	0.87mg SMX, 0.17mg TMP per ml

E. Use of Radioisotopes – Regulations regarding the use of radioisotopes in research animals at Rutgers can be found in the current Rutgers University Radiation Safety Guide (ipo.rutgers.edu/rehs).

VI. References

- Kassayova, E. et.al. 1999. Two-Phase Response of Rat Pineal melatonin to Lethal Whole-Body Irradiation with Gamma Rays. Physiol. Res. 48:227-230.
- Mihandoost, E, et al 2014. Consequences of Lethal-Whole-Body Gamma Radiation and Possible Ameliorative Role of Melatonin. The Scientific World Journal, Article ID 621570
- Zawaski et al 2017. Lethal and Sub-Lethal Irradiation of Sprague-Dawley Rats Causes Development of Dense Red Blood Cells. Blood 130 (Supplement 1), 4738.

RUTGERS UNIVERSITY IACUC Document #D1	Genotyping - Tissue Collection
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 4/13/2021 (version 3.0)

I. Purpose

This document describes acceptable tissue collection procedures for genotyping mice and rats.

II. Introduction

When tissue samples are required for genotyping of mice and rats, **investigators are encouraged to use the least invasive method possible to ensure animal welfare. If animal identification is being performed through the removal of a piece of tissue, that same sample of removed tissue should be used for genotyping purposes.** This avoids performing repeated invasive procedures on the same animal. Researchers should remove the least amount of tissue necessary to perform genotyping. Methods that do not permanently alter the animal or do not produce more than momentary pain should be prioritized. If tissues cannot be used for both purposes, justification for harvesting additional tissue for genotyping only must be provided in the protocol and approved by the IACUC.

Genotyping of animals should be performed prior to weaning to reduce retention of animals of an undesirable genotype or sex and reduce potential pain and distress associated with collecting tissue samples at older ages. Consult CMR veterinarians if problems with hemostasis are encountered or expected (e.g., mutant mice with clotting disorders).

III. Responsibilities

Any animal user at Rutgers University who is performing genotyping is required to follow the instructions in this document.

IV. Methods

A. Tools

1. Scissors, blades, and ear punches must be sharp. Usage of dull or rusted instruments is unacceptable.
2. Instruments must be sterilized before use, by one of the following methods:
   a. Autoclave
   b. Use hot bead sterilizer (allow instrument to cool before using on the animal)
   c. Soak in sterilant (such as chlorine dioxide solution) for at least 5 minutes
3. Instruments must be cleaned and disinfected between animals to minimize infection and DNA cross-contamination of the samples. It is recommended that instruments be sprayed with chlorine dioxide and rinsed with 70% isopropyl alcohol between animals.

B. Techniques

It is recommended to use the least invasive method of tissue collection possible. The procedures below are listed in increasing order of their relative invasiveness.

The first four methods do not require Institutional Animal Care and Use Committee (IACUC) approval:

1. **Feces –** Collect fresh identified stool pellets.

2. **Hair –** Gently pluck tufts of hair to include the follicle using tweezers or hemostats. Samples may be collected at the neck line between the shoulder blades. Animals should not have exposed patches of skin after collection, as only small tufts are needed.

3. **Buccal/Cheek Swab**
   a. Restrain mouse.
   b. Gently swab the inner surfaces of both cheeks with a sterile 2mm cotton-tipped applicator.

4. **Ear Punching or Notching**
   a. Restrain the animal.
   b. Using a clean, sharp ear punch, remove a circle of ear tissue (punching, typically 2mm in diameter) or slice a small (2-3mm) portion of the pinna with sharp scissors (snipping). This method can be performed on mice once the ears have developed (>8 days of age).
   c. When also used for identification, record sequence/coding (e.g. see IACUC Policy Rodent Identification).

All of the **other techniques are required to be included in an approved IACUC protocol:**

5. **Blood** - Blood collection for genotyping must be consistent with the method(s) described in the approved protocol. Total blood volume collection for genotyping should not exceed 1% of body weight.

6. **Tail Biopsy/Snip**
   Tail biopsy in mice and rats should be done between 8 and 21 days of age in order to yield the most tissue with the least amount of pain. It is strongly recommended to perform tail snips in rodents under 17 days of age. Tail biopsies performed after 21 days of age must be performed using anesthesia and/or post-operative analgesics as per the table below.

   a. Restrain the animal. Note, each animal should be identified (e.g., ear tag or punch) prior to tail snip, and individual animal ID recorded with tail sample.
   b. Disinfect tip of tail with 70% isopropyl alcohol (preferred) or ethanol.
   c. Using sterile sharp scissors or scalpel cut a **maximum of 5mm** from the tip of the tail. Only the minimum amount of tissue should be taken.
   d. If multiple samples are required, the total of tissue taken cannot exceed 5mm.
   e. Hemostasis must be obtained. Apply direct pressure (preferably with gauze) to the tip of the tail for up to 30 seconds. The animal must not be returned to the cage **until bleeding stops**.
   f. Summary of tail biopsy requirements:

age	anesthetic	analgesia	hemostasis
≤21 days biopsy ≤5mm	no	no	yes
22-28 days biopsy ≤5mm	yes	no	yes
≥29 days biopsy ≤5mm	yes	yes	yes
any age, biopsy ≥5 mm; Requires scientific justification	yes	yes	yes

g. General anesthesia
Use of a short-acting volatile anesthetic such as isoflurane is recommended; however, injectable anesthetics may be used. Closely monitor the animal's recovery from anesthesia. All anesthetics must be listed in an approved protocol.

h. Analgesia
Analgesics must be administered post-procedurally to any animals showing signs of pain or distress. Consider using pre-emptive analgesia e.g. buprenorphine, meloxicam, or carprofen, subcutaneously. Additional dosing may be required if animals show continued signs of pain. All analgesics must be listed in an approved protocol. Examples of acceptable analgesics can be found in the IACUC Policy Anesthesia and Analgesia in Laboratory Animals. Consult a CMR veterinarian for information on suitable drugs and doses.

7. **Toe Amputation/Clip**
Toe clipping is a method of tissue collection and small rodent identification that involves a numerical scheme in which only the most distal of the three phalanges of one toe per paw is removed.

Toe clipping is discouraged and only allowed with strong scientific justification and when also used for identification with the following conditions:

a. Rodents up to 7 days
b. Removal of only one toe per paw
c. Directions:
   i. Gently restrain the rodent.
   ii. Start with the toes of the hind feet as they are less sensitive than the front toes.
   iii. Wipe down the skin of the foot and digits with alcohol.
   iv. Use small, sharp, sterile scissors (such as iridectomy scissors).
   v. Extend the leg and remove distal phalanx.
   vi. Remove only the distal phalanx and remove the entire nail bed to avoid re-growth.
   vii. Do not cut the hallux (aka dew claw, thumb, little toe of the forepaw) as this may decrease the rodent's grasping ability.
   viii. Bleeding is usually minimal. If needed, use one of the following methods to control hemorrhage: 1. Light, direct digital pressure with gauze over the cut surface. 2. Medical-grade, non-toxic, styptic powder or gel (e.g., Clotisol, Kwik Stop®).

Consult a CMR veterinarian if problems with hemostasis are encountered or expected (e.g., mutant mice with clotting disorders).

RUTGERS UNIVERSITY IACUC Document #D2	Substance Administration and Blood Collection in Rodents
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 5/19/2021 (version 3.0)

I. Purpose

This document provides investigators with reference values related to the administration of substances and collection of blood in rodents via the most common experimentally used routes.

II. Introduction

The values included in this document are cited directly from the literature, represent an average of multiple cited sources, or are based on the professional experience of the Veterinary Staff. This document contains three sections: (1) recommendations regarding dosing of compounds based on common routes of administration. Included are the maximum recommended volumes for each route. (2) Common sites of blood collection, along with expected volumes for each site/method. (3) Injection of tumors/pellets through a trocar.

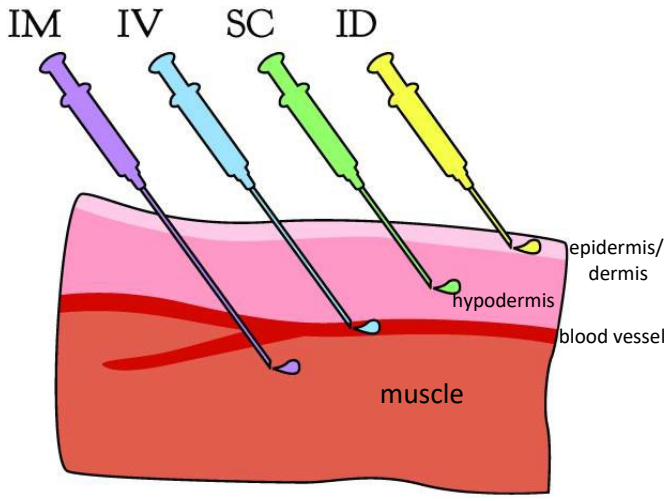
III. Responsibilities

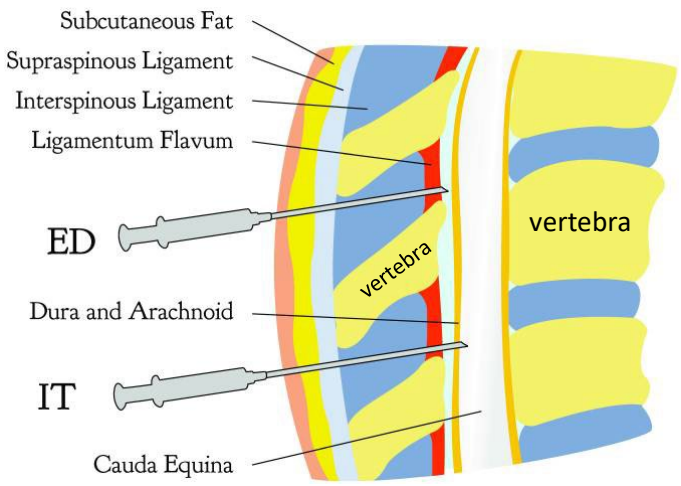
This document applies to all animal users administering substances or collecting blood from rodents at Rutgers University.

IV. Definitions

The following abbreviations will be used in this document:

PO = *per os* (oral gavage)	IM = intramuscular
IN = intranasal	IV = intravenous
SC = subcutaneous	IT = intrathecal (into subarachnoid space of spinal column)
ID = intradermal	EP/ED = epidural (outside of meninges)
IP = intraperitoneal	ICV = intracerebroventricular (into lateral ventricle of brain)
RO = retro-orbital	ITR = intratracheal





V. Methods

A. Fluid Administration - Vehicle selection is an important consideration in compound administration. Ideally, the vehicle should be biologically inert and have no toxic effect on the animal. Osmolality, pH and viscosity of the vehicle should be considered when preparing compounds. If possible, compounds should be prepared so that the delivery volume is below the maximum volume. Note that compounds cannot be delivered in a volume greater than the maximum value listed under body weight (highlighted in red) without prior IACUC approval. Please see the charts below.

Mice (values across top are body weights in grams)

All values listed on chart are in **milliliters (ml)**; value is the maximum volume allowed by that route.
* = requires additional justification

route	10	15	20	25	30	≥35	needle
PO	0.2	0.3	0.4	0.5	0.6	0.7	18-24ga
IN	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	n/a
SC (per site)	0.1	0.15	0.2	0.25	0.3	0.35	≥20ga
RO	0.01	0.05	0.10	0.10	0.15	0.15	≥25ga
ID	0.05	0.05	0.05	0.05	0.05	0.05	≥25ga
IP	0.5	<1.0	1.0	1.5	<2.0	2.0	≥21ga
IM*	0.05	0.05	0.05	0.05	0.05	0.05	≥23ga
EP	0.002	0.003	0.004	0.005	0.006	0.007	30ga
IT	0.001	0.001	0.002	0.002	0.003	0.004	30ga
ICV	≤0.003	≤0.003	≤0.003	≤0.003	≤0.003	≤0.003	30ga
IV (bolus)	0.05	0.075	0.10	0.125	0.150	0.175	≥25ga
IV (infusion, per hour)	0.025	0.0375	0.05	0.0625	0.075	0.0875	≥25ga
ITR	0.1	0.1	0.1	0.1	0.1	0.1	

Rats (values across top are body weights in **grams**)

All values listed on chart are in **milliliters (ml)**; value is the maximum volume allowed by that route.

route	100	150	200	250	300	350	400	450	500	needle
PO	2	3	4	5	6	7	8	9	10	16-20ga
IN	35	35	35	35	35	35	35	35	35	N/A
SC	0.5	0.75	1	1.25	1.5	1.75	2	2.25	2.5	≥20ga
RO	0.5	0.75	1	1.25	1.5	1.75	2	2.25	2.5	≥23ga
ID	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	≥25ga
IP	1	1.5	2	2.5	3	3.5	4	4.5	5	≥21ga
IM	0.005	0.0075	0.01	0.0125	0.015	0.0175	0.02	0.0225	0.025	≥21ga
EP	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	≥28ga
IT	0.01	0.015	0.02	0.025	0.03	0.035	0.04	0.045	0.05	≥28ga
ICV (bolus)	≤0.006	≤0.006	≤0.006	≤0.006	≤0.006	≤0.006	≤0.006	≤0.006	≤0.006	≥28ga
IV (bolus)	0.5	0.75	1	1.25	1.5	1.75	2	2.25	2.5	≥23ga
IV (slow)	2	3	4	5	6	7	8	9	10	≥23ga
ITR	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	

Notes on specific routes:

Oral gavage (PO) is performed using a feeding needle only (one that has an atraumatic, blunt ball at the end to prevent damage to the esophagus), appropriate needle length is determined by measuring from the mouth to the last rib; inject slowly. Proficient oral gavage should result in no significant animal losses (>95% survival rate).

Subcutaneous injections are limited to a maximum of 3 injections every 24hrs; typically SC injections are delivered on the back/dorsum.

-SC fluids to account for blood/fluid loss (such as given postoperatively) are typically <0.5ml in the mouse and <3ml in the rat and split between 2-3 sites.

Retro-orbital sinus/plexus injections are a good alternative to standard IV injections (such as through the tail vein). All RO injections must be performed under general anesthesia.

Intramuscular injections are limited to the quadriceps femoris or biceps femoris muscle groups. Due to the small size of mice, IM injections are strongly discouraged in mice, due to potential for nerve damage and muscle necrosis/irritation. No more than two IM injections are permitted every 24hrs for other rodents.

Intrathecal, Intracerebroventricular injections should be given over at least 1-2min per 10 µl in mice and no greater than 0.25ml per minute in rats.

Intratracheal injections must be administered at doses less than or equal to 50 µl in mice or 300 µl in rats. All intratracheal injections must be performed under heavy sedation or general anesthesia.

A **bolus Intravenous injection** is delivered within 1 minute or less; typical IV injection sites in rodents include the lateral tail veins and the saphenous veins.

A **slow Intravenous injection** is delivered over a 5-10 minute period.

For **Intraperitoneal injections** animals should be inverted with the head down, and injections should be given in the lower quadrant of the abdomen (with preference given to the lower right quadrant). Aspirate to verify needle is in the correct location.

Contact the Veterinary Staff for information regarding other routes of administration.

B. Blood Collection

Circulating blood volume (CBV) in rodents is ~55-70 ml/kg (~5.5-7.0% body weight, mouse average = 7.2% BW, rat average = 6.4% BW). Investigators can safely remove 1% CBV every 24hrs, or 10% CBV every 2 – 4 weeks. No more than 20% CBV can be removed at one time Animals MUST have appropriate recovery time after collection (see below), based on the total volume of blood removed.

Factors to consider when choosing the best blood collection method should include:

-type of sample (whole blood, serum, etc.)
-quantity of blood required
-quality of sample (sterility, tissue contamination, etc.)
-frequency of sampling
-health status of the animal(s)
-training/experience of collector

Blood sample volume ranges based on body weight:

	BW(g)	CBV (ml)	1% (ml)	10% (ml)	15% (ml)	20% (ml)
Mouse	20	1.10-1.40	0.011-0.014	0.11-0.14	0.165-0.21	0.22-0.28
	25	1.37-1.75	0.014-0.018	0.14-0.18	0.21-0.27	0.28-0.36
	30	1.65-2.10	0.017-0.021	0.17-0.21	0.25-0.315	0.34-0.42
	35	1.93-2.45	0.019-0.025	0.19-0.25	0.285-0.375	0.38-0.50
	40	2.20-2.80	0.022-0.028	0.22-0.28	0.33-0.42	0.44-0.56
Rat	125	6.88 - 8.75	0.069-0.088	0.69-0.88	1.032-1.3125	1.38-1.76
	150	8.25-10.50	0.082-0.105	0.82-1.0	1.236-1.575	1.64-2.0
	200	11.00-14.00	0.11-0.14	1.1-1.4	1.65-2.1	2.2-2.8
	250	13.75-17.50	0.14-0.18	1.4-1.8	2.063-2.625	2.8-3.6
	300	16.50-21.00	0.17-0.21	1.7-2.1	2.475-3.15	3.4-4.2
	350	19.25-24.50	0.19-0.25	1.9-2.5	2.886-3.675	3.6-5.0

Blood collection recovery times:

single sample		**multiple samples**	
% CBV removed	recovery period	% CBV removed in 24 hours	recovery period
1%	24 hours	1%	24 hours
5%	1 week	5%	1 week
10%	2 weeks	10%	2 weeks
20%	4 weeks	20%	4 weeks

Blood collection sites in rodents:

		general anesthesia?	**repeat samples?**	**expected volume**	**tissue damage**	**device/ needle**
mouse (25g)	retro-orbital	yes	limited	5% CBV	mod/high	cap tube
	Submandibular/facial vein (adults only)	no	yes	0.2-0.4ml	mod	lancet, 3-5mm
	saphenous	no	yes	5% CBV	low	25-27ga
	lateral tail vein	no	yes	0.1-0.15ml	low	25-27ga
	ventral tail artery	no	yes	0.1-0.2ml	low	25-27ga
	cardiac	yes/terminal	no	50% CBV	mod	23ga
rat (300g)	Submandibular/facial vein	no	yes	0.2-0.5ml	mod	lancet, 5-8mm
	sublingual	yes	yes	0.2-1ml	low	23-25ga
	jugular	no	limited	5% CBV	low	25-27ga
	saphenous	no	yes	5% CBV	low	25-27ga
	lateral tail vein	no	yes	up to 2ml	low	25-27ga
	ventral tail artery	no	yes	0.1-0.2ml	low	25-27ga
	cardiac	yes/terminal	no	50% CBV	mod	23ga

Notes on specific techniques:

Mandibular samples will contain a mixture of venous and arterial blood.

Blood from the **saphenous** and **tail veins** can be achieved either by introducing an appropriate needle into the vessel, or by nicking the vessel and collecting blood into a container; note that samples collected by the latter method will not be sterile and could be contaminated with tissue(s).

Lateral tail vein: Prewarming the tail under a heat lamp or local warming will cause vasodilation, increasing yield.

Retro-orbital samples are collected with a heparinized capillary tube from the medial/rostral canthus of the eye only. The animal must be anesthetized. For chronic studies, samples can be taken at a maximum of once a week from the same eye. Personnel must be adequately trained in technique to avoid injury to the animal. **Retro-orbital bleeds are not permitted on rats.**

Cardiac blood sampling is only permitted as a terminal procedure in a deeply anesthetized animal.

C. Tumor/Pellet Delivery by Trocar

Pieces of tumor and pellets (often for slow release of drugs or hormones) are usually implanted subcutaneously in rodents through a large-bore needle called a trocar. Because of the large diameter of a trocar, more than momentary pain is associated with their use; therefore, all procedures involving trocars are considered minor survival surgery by the IACUC. Animals MUST be under general anesthesia and have a local anesthetic agent applied at the trocar site for these procedures. Trocars will cause more damage to the skin compared to smaller gauge hypodermic needles, and skin closure (suture, staples, wound clips, surgical glue) may be needed post injection. Additionally, animals should be provided with at least one dose of postoperative analgesia.

Common sites of tumor/pellet insertion include the lateral flank (just in front of the hip) and the intrascapular area of the dorsum (between the shoulder blades). The IACUC recommends injection of tumors at the lateral flank to reduce irritation from the overlying wire insert of the cage (can irritate developing tumors on the dorsum). For recommendations regarding other acceptable areas, please contact CMR veterinary staff.

RUTGERS UNIVERSITY IACUC Document #D3	Food and Fluid Restriction/Regulation
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 3/17/2021 (version 3.0)

I. Purpose

The purpose of this document is to provide animal users with guidelines regarding withholding of food and/or water from laboratory animals. Food and fluid restriction/regulation includes any deviation from normal husbandry procedures (*ad lib* food and water). This document excludes certain routine, clinical situations under the direction of the veterinarians such as altered diets that do not involve restriction/ regulation (high / low fat diet, medicated food / water, etc.) or withdrawal of food prior to surgery for certain species to prevent emesis under general anesthesia (usually <12hrs).

II. Introduction

The Guide for the Care and Use of Laboratory Animals (2011) requires careful monitoring of animals that are on food or fluid regulation/restriction -

"Regulation of food or fluid intake may be required for the conduct of some physiological, neuroscience, and behavioral research protocols. The regulation process may entail scheduled access to food or fluid sources, or restriction, in which the total volume of food or fluid consumed is strictly monitored and controlled. The objective when these studies are being planned and executed should be to **use the least restriction necessary** to achieve the scientific objective while maintaining animal well-being." - Guide pages 30-31

"The animals should be closely monitored to ensure that food and fluid intake meets their nutritional needs. **Body weights should be recorded at least weekly and more often for animals requiring greater restrictions. Written records should be maintained for each animal to document daily food and fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol.** In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended." - Guide page 31

Common experimental situations that may require food or fluid restriction/regulation include:

- Behavioral research protocols in order to train animals to perform a task, while providing food or water as a reward for the correct behavior
- Nutrition studies
- Food restriction is common for some sedentary laboratory species in order to control obesity or maximize lifespan (such as maintaining 85% of body weight)

Generally, an eight-week old mouse consumes 6.7ml water and 5g food in 24 hours; a rat consumes 8-11ml water/100g body weight and 5g food/100 gram body weight in 24 hours. However, there are significant variations in fluid intake based on strain, sex and age. In-house ad lib intake should be determined for the strain, sex, age, and weight of rodents used for the study; published values may be used in lieu of in-house determination, if available.

III. Responsibilities

This document applies to any experimental situation at Rutgers University that involves restriction/ regulation of food and/or fluid to laboratory animals, excluding situations previously described in the 'Purpose' section of this document.

IV. Definitions

ad libitum (ad lib) – freely available

fasting – the removal of food prior to an experimental manipulation such as surgery, imaging, or metabolic testing; duration is typically less than 12hrs

food/fluid regulation – animals have scheduled access to food/fluid such that an animal can consume as much as desired at regular intervals

food/fluid restriction – the total volume of food/fluid is strictly controlled and animals will consume less than the amount desired

V. Methods

A. Minimum Requirements for Protocols that involve Food and/or Fluid Restriction/Regulation

The following items are the minimum requirements for all protocols that use food or fluid restriction/regulation:

1. All protocols that require the use of food or fluid restriction/regulation must be justified. Include a description of procedures to be used to monitor animals on food or water restriction/regulation and scientific justification.
2. The maximum period of restriction/regulation must be clearly stated in the protocol.
3. Weight loss of greater than 20% requires that the animal be removed from the restriction/regulation, unless specifically justified in the protocol. Weight loss is relative to the starting body weight in adult animals. For young, growing animals, growth must be taken into account when determining predicted body weight. Weight logs must be kept in the animal room.
4. Animals on fluid regulation/restriction must be monitored for daily intake and assessed for dehydration by an experienced observer. Record daily intake of fluids and assessment of hydration (e.g. skin tenting) on the attached log sheet. Individual cages must be marked using the Food/Fluid Restriction cage card below.
5. Establish behavioral and clinical changes to be used as criteria for the temporary or permanent removal of an animal from the experimental protocol. Describe the criteria in the appropriate protocol.

B. Procedures

Food or water restriction/regulation must be justified based on the scientific objectives of the study; the least amount of restriction/regulation that will achieve the objectives must be used.

1. Baseline body weight of animal(s) must be measured before food or water restriction/regulation is begun.
2. For young, growing animals, desired body weight is determined by comparison to a control animal's body weight or to published expected growth curves (accounting for species/strain, age, sex, etc.).

3. Restriction must be based on a measurable parameter such as percentage of *ad lib* intake or duration of restriction.
4. In the case of operant response protocols, the use of a highly preferred food or fluid as positive reinforcement is recommended over restriction/regulation.
5. When using fluid rewards as motivation for task performance, it is imperative for the investigator to ensure that the daily requirements to maintain a healthy state are met by the sum of earned rewards and supplemental fluid offered.
6. Initially body weight must be measured at least 3 times during the first week for any animal on food or water restriction/regulation, then weekly thereafter.
7. For animals on lifetime food restriction (such as maintaining 85% body weight), animals must be weighed at least 2-3 times weekly for the first month of the study to ensure plateau of weight loss, then only need to be weighed once weekly thereafter.
8. Hydration status of water restricted/regulated animals must be assessed by laboratory researchers daily, including weekend and holidays.
9. Experimental endpoints, clinical symptoms, and conditions for temporary or permanent removal of an animal from the study must be described in the IACUC application. Examples include: body weight loss, appearance (sunken eyes), behavior (lethargic, listless) and other health issues, and failure of growing animals to gain weight.

    a. For **food restriction/regulation**: a rodent may not lose more than 20% of body weight of age-strain-sex matched animal.
        i. After 20% weight loss has been achieved (the animal weight is 80% of baseline weight or matched controls), the daily food allowance must be increased to prevent additional weight loss.
        ii. Restriction/regulation cannot be attempted again until the animal weighs at least 80% of its original weight. A rodent given a body condition score of two or lower must have its daily food allowance increased. Increase the food ration until the animal receives a body condition score of 2.5 or higher.

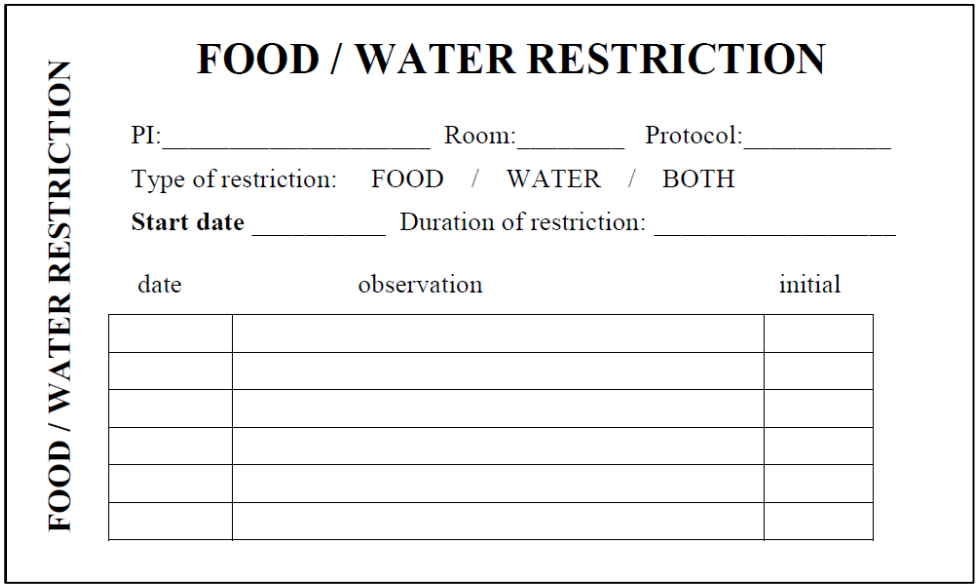
    b. For **fluid restriction/regulation:**
        i. Rodents on fluid restriction/regulation with a weight loss of 10% of baseline weight and are considered clinically dehydrated by CMR staff and should be allowed ad lib access to water until the hydration status returns to normal. In addition, 0.5-1ml (mouse) or 2-3ml (rat) of subcutaneous lactated ringer's solution or isotonic saline (0.9% NaCl) must be administered and the Veterinary Staff consulted.
        ii. Under no circumstances should laboratory staff remove water valves for water restriction studies. All animals undergoing water restriction studies must be removed from IVC racks, unless otherwise approved by a CMR supervisor.

10. Individual cages must be marked with the "food / water restriction/regulation" card at the end of this document. Contact the Facility Supervisor for details.
11. A daily log sheet must be maintained for each cage on restricted/regulated food or water protocols and kept with the animals in the animal holding room. All investigators are required to use the log sheet at the end of this document. Contact the Facility Supervisor for details.
12. Research staff is responsible for monitoring animals on food or fluid restriction/regulation studies.

VI. References

- The Guide for the Care and Use of Laboratory Animals, current version

Appendix I – Cage Card for Food/Fluid Restriction



FOOD / WATER RESTRICTION

FOOD / WATER RESTRICTION

PI:_____ Room:_____ Protocol:_____

Type of restriction: FOOD / WATER / BOTH

Start date _____ Duration of restriction: _____

date	observation	initial

Appendix II – Record Form for Rodent Food/Fluid Restriction/Regulation

Rutgers Rodent Food/Fluid Restriction/Regulation Record Form

RUTGERS UNIVERSITY

Protocol number:	Species: Mice Rats Other:
Principal Investigator:	Restriction: Food Water Both
Laboratory member:	Duration of restriction/regulation:
Laboratory phone:	Reason for restriction/regulation: _____
After hours phone:	_____
Has this restriction been approved by the IACUC? Y / N **If 'no' or unsure contact IACUC before proceeding**	

Date	Animal ID	Body wt (g)	Time food or water removed	Time food/water offered	Skin turgor [1]	Comments	Initial

RUTGERS UNIVERSITY IACUC Document #D4	Autophagia and Autotomy (Self-mutilation)
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 3/17/2021 (version 3.0)

I. Purpose

The purpose of this document is to generate a procedure for a scoring and treatment system for investigators that perform experiments which may result in self-mutilation by test subjects.

II. Introduction

Animals that receive neurological lesions affecting the somatosensory system will often injure the affected areas/dermatomes through a process of self-mutilation. This typically occurs after damage to either the central or the peripheral nervous system. The cause of this condition is not fully understood.

III. Responsibilities

This document applies to any animal user that performs experiments that could result in self-mutilation by test subjects.

IV. Definitions

Autophagia - self-mutilation of a region of the body

Autotomy - self-amputation of limbs, digits, or a portion of a single digit

V. Methods

There are currently no generally accepted or effective therapies for neuropathy. The incidence and severity in rodents is highly variable, strain-dependent, housing-dependent, and sex-dependent. First-line treatments usually consist of either anticonvulsant drugs (such as gabapentin or carbamazepine) or antidepressant drugs such as tricyclics and serotonin/noradrenaline reuptake inhibitors. Traditional analgesics such as morphine have been shown to exacerbate the condition and prolong recovery.

Self-mutilation scoring:

Autotomy: Any animal that self-amputates must be euthanized immediately.

Autophagia is scored using the following scale:

0 = no signs of autophagia
1 = loss of hair in region (underlying skin is normal or mildly inflamed but no penetration)
2 = exposure of subcutaneous layers of skin
3 = exposure of underlying skeletal muscle
4 = penetration through skeletal muscle
5 = exposure of internal organs and/or bone

Treatment (rodents only):

Any rodent that experiences a procedure that could result in self-mutilation should be watched daily for the first seven days post-operatively. The Institutional Animal Care and Use Committee (IACUC) suggests (but does not require) placement of an Elizabethan collar (E-collar) and the initiation of oral acetaminophen (a minimum of 64mg/kg once daily) for one week postoperatively if possible. Over the counter pediatric syrup is usually palatable to rodents and can be given by mouth via a syringe (no needle). Acetaminophen CANNOT be provided via drinking water. Additionally, topical preparations are commercially available (Chew Guard, New Skin to avert/treat the condition.

If not treated preemptively, at the first sign of **autophagia** (score of 1-2), the animal MUST be treated with oral acetaminophen (Tylenol, 64mg/kg once daily) and may be outfitted with an E-collar or euthanized. At level 3, animals require surgical repair (which must be described in the experimental protocol), along with continued oral acetaminophen. If autophagia continues or progresses for 3 or more days after initiation of treatment, the animal must be euthanized. **Animals scoring a 4 or 5 must be euthanized immediately.**

Consult CMR veterinarians when further guidance is needed.

Treatment (non-rodents):

Consult CMR veterinarians during protocol development and for any unforeseen complications.

RUTGERS UNIVERSITY IACUC Document #D5	Rodent Identification
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 2/17/2021 (version 3.0)

I. Purpose

The purpose of this document is to provide guidelines on identification of live rodents. This policy only applies to laboratory mice and rats. For identification of all other species, contact CMR.

II. Introduction

Identification of live animals is critical to the efficient pursuit of research and reducing the number of animals involved in a research project. Tattoos, colored stains, subcutaneous transponders (microchips) and the use of numbered ear tags are common methods of identification. If animal identification is being performed through the removal of a piece of tissue, that same sample of removed tissue should be used for genotyping purposes. Methods that do not permanently alter the animal or produce slight momentary pain should be prioritized. If tissues will/cannot be used for both purposes, justification for removing additional tissue must be provided in the protocol and approved by the IACUC.

III. Responsibilities

All researchers that generate heterozygous or hemizygotic progeny through a breeding scheme are required to use genotyping and identification procedures to minimize the number of unwanted progeny generated and the wasting of an experimental animal because of inadequate records/identification. Rodents should have an easily read method of identification to ensure the correct animal is used for each experimental protocol. Cage cards must be used for every rodent cage; however, relying on cage card level identification alone may result in misidentification of individual animals. Minimum cage card information must include: source of animal, strain/stock, names and contacts of PI, arrival date, birthdate, protocol number and genotype information when applicable (see *Guide* p. 75).

IV. Methods

I. **Ear punches/notches/clipping:** A number of variations using either ear punches or notches can be employed for animal identification. Most involve marks on one or both ears. The tissue from these procedures should be used for genotyping, if genotyping is also necessary. The disadvantages of ear marking are future tearing of the ear due to fighting or scratching and the potential for tissue regrowth as the animal ages, obliterating the marking, and in both cases obscuring the identification. No age restrictions apply for performing this procedure.

II. **Ear tags:** Commercially available ear tags can be used to identify animals and have the advantage that there are unlimited unique numbers available. Tags are positioned at the lateral base of the ear, approximately 3mm from the edge of the ear pinna. The disadvantage of this approach is that ear tags are difficult to secure on very small mice and can be torn from the ears with scratching and fighting. While there are no age restrictions for attaching ear tags, CMR recommends ear tagging around or after weaning age.

III. **Tattoos:** Tattooing can be used both on neonates and adults as a permanent means of identification. Anesthesia is not required but may aid with restraint. EMLA cream or local anesthetic spray may be used as local anesthetics prior to tattooing. Systems are available to tattoo either toes or tails. There are no age restrictions for tattooing an animal.

IV. **Colored stains:** Non-toxic, indelible markers/dye can be used to color the fur or tail of an animal temporarily. There are no age restrictions for color staining an animal's skin or fur.

V. **Toe clipping:** Toe clipping is discouraged and only allowed with strong scientific justification and when also used for genotyping with the following conditions:
   1. Rodents up to 7 days
   2. Removal of only one toe per paw
   3. Directions:
      a. Gently restrain the rodent.
      b. Start with the toes of the hind feet as they are less sensitive than the front toes.
      c. Wipe down the skin of the foot and digits with alcohol.
      d. Use small, sharp, sterile scissors (such as ocular microsurgical scissors).
      e. Extend the leg and remove distal phalanx.
      f. Remove only the distal phalanx and remove the entire nail bed to avoid re-growth.
      g. Do not cut the hallux (aka dew claw, thumb, little toe of the forepaw) as this may decrease the rodent's grasping ability.
      h. Bleeding is usually minimal. If needed, use one of the following methods to control hemorrhage:
         - Light, direct digital pressure with gauze over the cut surface
         - Medical-grade, non-toxic, styptic powder or gel (e.g., Clotisol, Kwik Stop®)

         Consult the veterinarians if problems with hemostasis are encountered or expected (e.g., mutant mice with clotting disorders).

VI. **Subcutaneous transponders (microchips):** Larger species and rodents 21 days or older can be implanted with commercially available microchips or transponders that can be scanned using an electronic hand held device. This is a relatively expensive option, although the units can be recovered and reused after sterilization. Because of the large diameter of a trocar (≤14ga), animals must be under general anesthesia. Trocars will cause more damage to the skin compared to smaller gauge hypodermic needles, and skin closure (suture, staples, wound clips, surgical glue) may be needed post-injection. Animals may be provided with analgesia postoperatively. Subcutaneous transponder implantation should be performed around the time of weaning or older.

VII. Summary of identification methods:

Identification Method	Age Restriction	Anesthesia Necessary	Analgesia Necessary	IACUC Approval Necessary	Additional Information
Ear notch/punch/clip	None	No	No	No	• Punch or scissors must be disinfected between animals. • Tissue can also be used for genotyping. • Repeat punching may be necessary if tissue re-seals.

Ear tag	Recommended to perform around or after weaning age	No	No	No	• Proper ear tagging technique is necessary to avoid inflammation, infection, and pressure necrosis • Ear tags may not be compatible with protocols involving advanced imaging (MRI, CT)
Tattoo	None	Not required but recommended	No	Yes§	• Site should be disinfected prior to use.
Colored stain	None	No	No	No	• Site may need to be marked repeatedly.
Toe clip	Up to 7 days of age	No	No	Yes	• Instruments must be sterilized before use and disinfected between animals. • Only one toe from each paw may be cut (a maximum of 4 toes cut in each animal). • Hemostasis must be achieved. • May impair grip strength.
Subcutaneous transponder (microchip)	Recommended to perform at or after weaning age	General anesthesia necessary	No	Yes	• May not be compatible with protocols involving advanced imaging (MRI, CT).

§ vendor placed tattoos are exempt

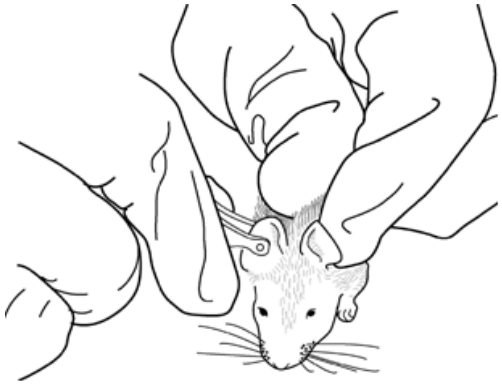


Figure 1. Appropriate restraint of mouse and placement of ear punch device.

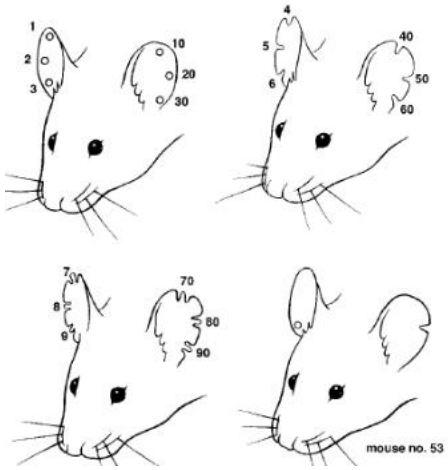


Figure 2. Commonly used numbering scheme for mouse ear punches/notches. From AALAS Learning Library (http://www.aalaslearninglibrary.org)

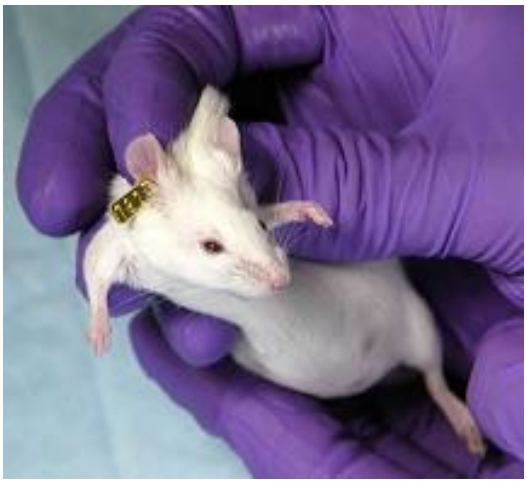


Figure 3. Appropriate positioning of ear tag.

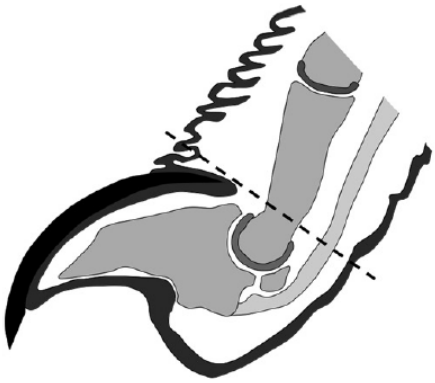


Figure 4. Appropriate technique for toe clipping requires removal of the entire most distal toe bone (3rd phalanx) and nail bed, which often requires removal of a small portion of the 2nd phalanx (adapted from Dahlborn, et al., 2013).

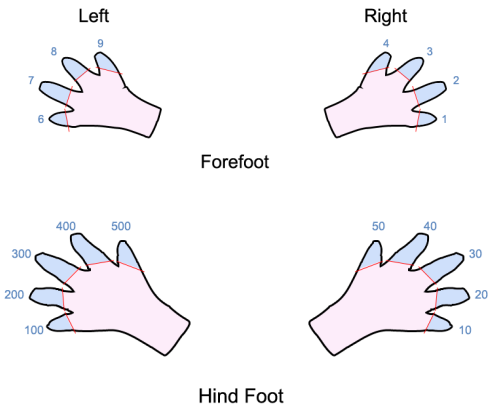


Figure 5. Commonly used numbering scheme for mouse toe clipping. Note: CMR requires starting with hind feet.

From Labfinity. (http://support.labfinity.com)

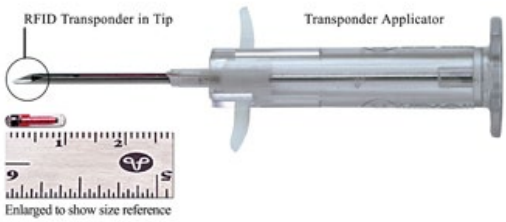


Figure 6. Commonly used transponder and applicator. From Kent Scientific. (http://www.kentscientific.com)

RUTGERS UNIVERSITY IACUC Document #D6	Prolonged Restraint
	Date Issued: 2/19/2020 (version 1.0) **Date Revised:** 3/17/2021 (version 2.0)

I. Purpose

The purpose of this document is to provide guidance on the use of prolonged restraint in conscious animals.

II. Introduction

Restraint is the use of physical means to limit some or all of an animal's normal movement for the purpose research or teaching. Restraint that lasts 15 minutes or longer in an unanesthetized animal is considered prolonged restraint. Prolonged restraint in conscious animals is potentially stressful and may only be used when essential for achieving research objectives. It must be detailed in the research protocol, reviewed, and approved by the IACUC. It is advisable to consult a CMR vet for guidance prior to submitting a protocol or amendment with prolonged restraint. This is especially helpful if your lab has not had recent experience with prolonged restraint at Rutgers.

III. Responsibilities

All animal users are required to follow these guidelines to minimize unnecessary pain or distress to the animal. The purpose of the restraint and its duration must be clearly explained to personnel involved with the study.

IV. Guidelines

- Restraint devices should not be considered a normal method of housing and must be justified in the animal use protocol.
- Do not use restraint devices simply as a convenience in handling or managing animals.
- Consider alternatives to physical restraint.
- Keep the period of restraint to the minimum required to accomplish the research objectives.
- Provide positive reinforcement training, when possible, to animals to be placed in restraint devices to assist in adaptation to the equipment and personnel. This training must be documented.
- Remove animals from study that fail to adapt to the restraint.
- Observe the animal at appropriate intervals, as determined by the IACUC.
- Veterinary care must be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change necessitates the temporary or permanent removal of the animal from restraint.

Examples of devices commonly used for prolonged restraint (including but not limited to)

- Panepinto slings
- Head fixation
- Nose cone inhalation
- Hind limb unloading

RUTGERS UNIVERSITY IACUC Policy #D7	Special Food and Water Formulations
	Date Issued: 6/16/2021 (version 1.0) **Date Revised:** n/a

I. Purpose

This policy provides guidance when animals are provided special (i.e., non-standard) food and/or water formulations. Note: food and fluid restriction (either standard or special food/water) is covered in the IACUC Policy Food and Fluid Restriction/Regulation.

II. Introduction

Researchers may need to provide animals with special food/water, typically to deliver compounds/drugs, induce metabolic pathology, or as part of supportive care (e.g., antibiotics provided in water post irradiation). Because some species are neophobic (avoiding novel substrates), they may be initially less likely to consume medicated diets/water. Additionally, some compounds are unstable or render the diet/water susceptible to spoilage. These factors must be considered when providing special formulations to animals.

III. Responsibilities

Provision of special food/water must be described in the IACUC protocol. Changing frequency should be included in the protocol. The research staff listed on the IACUC protocol are responsible for animal feeding and/or watering if special food/water is being provided unless arrangements are made with CMR staff. Food must be changed/refilled by research staff on a regular basis, depending on the formulation; storage of special formulations must comply with vendor specifications. Water must be changed/refilled by research staff at least once every three days. PIs should consult with a CMR veterinarian if they are unsure if a diet or water is considered special.

If research staff is providing special water in addition to standard water (e.g., a preference study where the animal can choose between standard water and sucrose water) and standard water is always available, this policy is not applicable. The special water must be labeled with the substance that has been added to it.

Gel diet/water is often provided as supportive care under various circumstances such as weaning and during the post-operative period. Provision of gel food/water for supportive care is exempt from this policy.

IV. Definitions

Special food and water – Any food and/or water containing added compounds (by the PI or vendor) such as antibiotics, anti-inflammatories, gene-induction compounds, sucrose, etc. or have non-standard proportions of routine ingredients (such as high-fat diets).

Standard diet – Any diet that is routinely provided to animals by CMR staff.

V. Methods

Each cage that receives special food/water must be marked with a special food/water cage card (available through the facility supervisor).

- **For special food**: the card must include at the minimum, the diet name, the date the special diet was started, and refill dates.
- **For special water**: the card must contain at the minimum, what has been added to the water, the date the special water was started, change frequency, and refill dates.

Alternatively, a log including the above information may be kept in the room. This can be used when all cages on a rack are on the same special food/water. Each cage, however, must still be identified with a blank special food/water cage card.

Animal users are encouraged to specify the location of the special food. If a special diet requires refrigeration or other special handling, the PI must coordinate with the facility supervisor for proper storage. Care must be taken by the PI to ensure that expired diets are discarded and replaced as needed.

Acclimation: When possible, special diet/water should be introduced to the animals for 3-5 days prior to onset of the experiment to ensure consumption.

Hazardous food/water: If a special diet/water is deemed hazardous (e.g., cuprizone diets), REHS must be consulted prior to use and the facility supervisor must be informed. Any REHS recommendations regarding the special diet/water (including appropriate signage) must be followed by research and CMR staff.

Note: Research staff must inform and consult with the facility supervisor to review the addition of special water (including the start time/date and end time/date), prior to beginning the study. Under no circumstances should laboratory staff remove water valves for special water studies. All animals undergoing special water studies must be removed from IVC racks, unless otherwise approved by a CMR supervisor.

If animals are on special food/water and care staff find these cages without food/water, CMR will make a reasonable attempt to contact the research staff regarding refill. If CMR cannot reach research staff, however, they must place standard food/water in the cage in the interest of animal welfare.

RUTGERS UNIVERSITY IACUC Document #E1	Evaluation of Cell Lines and Rodent-Derived Biologicals
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 3/17/2021 (version 3.0)

I. Purpose

The purpose of this document is to provide investigators with requirements for proper testing of biologicals that have not been tested for and documented free of murine pathogens. Evaluation of pathogen status is part of the rodent health surveillance program at Rutgers University to prevent the introduction of infectious diseases into animal facilities.

II. Introduction

Cell lines, tissues, and body fluids that have been derived from or passed through rodents may harbor infectious agents and contaminate in-house rodent colonies causing large scale, costly, deleterious effects to the animal research program and human health. Transplantable tumors, hybridomas, cell lines, blood products, and other biological materials (e.g., Matrigel) can be sources of both murine and human viruses that can contaminate rodents or pose serious risks to laboratory personnel. Testing is available to monitor microbiologic contamination and must be considered before introducing such material into animals.

III. Responsibilities

Information on the proposed use of cell lines/biologicals must be provided on the Institutional Animal Care and Use Committee (IACUC) application. The investigator is responsible to ensure all cell lines as described in IV.A below are tested and confirmed negative prior to use. For guidance on testing requirements and/or interpretation of results, CMR veterinarians should be consulted. Test results must be made available to the CMR and/or the IACUC upon request. Addition of new cell line(s) or biological products for ongoing approved projects also requires testing, review, and approval prior to use. All human cell lines, tissues, and biologics need to be registered with the Institutional Biosafety Committee (IBC) before use in animals. Contact biosafety@rutgers.edu for guidance.

IV. Methods

A. Products requiring testing regardless of source for each newly acquired batch:

1. All rodent cell lines and rodent derived biologicals that are administered to rodents.
2. Any cell lines passed through rodents, including human cell lines.
3. Rodent body fluids (blood and serum), cells, and tissues obtained from sources that have not been documented free of murine pathogens and intended for use in rodents at Rutgers University. This includes rodent sera for use in cell cultures.

Notes: Agents must be tested before introducing into the animals. If the cell lines or other biological materials are derived from in-house (Rutgers) rodents, testing is not required.

B. Minimal list of pathogens to be excluded. Contact CMR for additional questions.

1. **Mice** – Materials to be injected into mice **must test negative** for all of the following pathogens:

Mycoplasma pulmonis
Sendai virus
Mouse hepatitis virus
Minute virus of mice
Mouse parvovirus (MPV1-5)
Theiler's murine encephalomyelitis virus
Murine norovirus
Reovirus 3
Mouse rotavirus
Ectromelia virus
Lymphocytic choriomeningitis virus
Polyoma virus
Lactate dehydrogenase-elevating virus
Mouse adenovirus (MAD1, MAD2)
Hantavirus
C. bovis

2. **Rats** - Materials to be injected into rats **must test negative** for all of the following pathogens:

Mycoplasma pulmonis
Pneumonia virus of mice
Kilham's rat virus
Toolan's H1 virus
Rat parvovirus
Lymphocytic choriomeningitis virus
Rat cytomegalovirus
Sendai virus
Rat coronavirus
Sialodacryoadenitis virus
Rat minute virus
Seoul virus
Mouse adenovirus
Reovirus 3
Rat theilovirus

C. Recommended Testing Laboratories:

Charles River Laboratories Cell Line / Research Biologics Screening

Phone: 1.877.CRIVER.1 (1.877.274.8371)

Mouse Essential Panel or Rat Essential Panel
http://www.criver.com/files/pdfs/research-models/rm_ld_d_rodent_viral_pcr_for_research_biologics.aspx

Other laboratories may be used but must be pre-approved by the CMR Director or designee.

RUTGERS UNIVERSITY IACUC Document #E2	Drugs and Materials
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 3/17/2021 (version 3.0)

I. Purpose

The purpose of this document is to instruct investigators in the proper use, storage, and documentation of drugs (including controlled substances and non-pharmaceutical grade compounds) and the acceptable use of expired medical materials (syringes, suture, needles, etc.).

II. Introduction

Any substances administered to any animal must be pharmaceutical grade unless scientifically justified and approved by the IACUC.

III. Responsibilities

All substances and materials used for animal experimentation, teaching or training must be approved by the IACUC.

IV. Definitions

Substance is defined as anything administered to an animal including but not limited to a drug, pharmaceutical, medication, antibiotic, experimental and non-experimental food additive or supplement, physiological solution, biological, or chemical.

Material includes, but is not limited to, medical implements and devices such as sponges, gauze pads, suture, needles, surgical packs, scalpel blades, drapes, and catheters.

Pharmaceutical Grade Substances are drugs, biologics, or reagents that are approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary (USP-NF), or British Pharmacopeia (BP).

1. Pharmaceutical preparations are prepared in a sterile manner, have a demonstrated efficacy, a known concentration, and an established shelf life and expiration date. They have been determined to be appropriate for specified routes of administration (e.g. with respect to vehicle, pH, pyrogens, etc.). Injectable pharmaceutical drugs are generally packaged in an injection vial which helps maintain sterility and facilitates syringe loading. In many cases, pharmaceutical grade drugs are no more expensive than chemical grade drugs and they are generally more convenient. Examples of drugs that are available in both forms include pentobarbital and ketamine. In general, pharmaceutical grade drugs are distributed by veterinary or human medical supply houses (e.g. Henry Schein, Patterson Veterinary Supply, MWI Animal Health, Covetrus) whereas chemical grade drugs are sold by chemical companies such as Sigma-Aldrich.
2. Identification and Assistance in Substance Identification. A listing of pharmaceutical-grade drugs and biologics is available through the following databases:
   a. The Orange Book, the reference for FDA-approved human drugs http://www.accessdata.fda.gov/scripts/cder/ob/
   b. The Green Book, the reference for FDA-approved veterinary drugs. http://www.fda.gov/animalveterinary/products/approvedanimaldrugproducts/default.htm

Non-Pharmaceutical Grade (NPG) or Chemical Grade Substances are substances that have not been tested by the USP to assure identity and potency. Material that is not tested by these methods to meet those specifications is not eligible to be called pharmaceutical grade, or USP.

Veterinary Compounding is the customized manipulation of an approved drug by a veterinarian, or by a pharmacist upon the prescription of a veterinarian, to meet the needs of a research study. Institutional Animal Care and Use Committees (IACUCs) considering the use of veterinary compounding for research purposes are advised to consult the website below for more information about federal regulations. See: https://www.avma.org/KB/Policies/Pages/Compounding.aspx

Controlled (Scheduled) Substances are drugs and other substances regulated by the Controlled Substances Act (CSA) and enforced primarily through the Drug Enforcement Agency (DEA). Controlled substances are divided into five schedules (I-V) based on whether they have a currently accepted medical use in the United States and their abuse potential. See: CMR Controlled Substance Standard Operating Procedure and University policy: https://policies.rutgers.edu/90-2-3-currentpdf

V. Methods

A. Preparation of Substances and Materials

Substances and materials must be prepared according to manufacturer instructions and any compound-specific guidelines issued by a CMR veterinarian.

B. Storage of Substances and Materials

Substances and materials must be stored as per the manufacturer instructions, known laboratory practices, and any compound-specific guidelines issued by the IACUC and/or CMR.

C. Secondary Containers

1. Container Choice

a. Sterile empty injection vials are recommended and are available from CMR and commercial veterinary suppliers such as Fisher Scientific and Patterson Veterinary Supply.

https://www.pattersonvet.com/Search?q=Sterile+Empty+Vial

https://www.fishersci.com/shop/products/depyrogenated-sterile-empty-vials/p-4526493

b. Sterile Falcon and Eppendorf tubes are not recommended for mixing and storing substance aliquots. If they must be used, preparation must occur in a laminar flow hood using appropriate aseptic technique.

2. Labeling of Substances Transferred to Another Container

When substances or mixtures are transferred from the original vendor container to a secondary storage vessel, the vessel must be legibly labeled with the bulleted items below. If there is inadequate space on the label, the additional information must be provided on a separate sheet that accompanies and is stored with the chemical or mixture.

- Name of substance
- Date of preparation or transfer
- Expiration date (See *Expired Substances and Materials* section below)

- Concentration(s) of drug(s)
- Initials of the person who transferred the drug

D. Non-Pharmaceutical Grade Compounds

1. Justification of Use for Non-Pharmaceutical Grade Compounds.

a. "The use of pharmaceutical-grade chemicals and other substances ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals. They should therefore be used, when available, for all animal-related procedures. The use of non-pharmaceutical-grade chemicals or substances must be described and justified in the animal use protocol and be approved by the IACUC; for example, the use of a non-pharmaceutical-grade chemical or substance may be necessary to meet the scientific goals of a project or when a veterinary or human pharmaceutical-grade product is unavailable. In such instances, consideration must be given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use."[1] **Cost savings is not an adequate justification for using non-pharmaceutical-grade compounds.**

b. To secure approval for the use of non-pharmaceutical grade substances, the PI must:

i. provide sound scientific justification for the use of the compound,
ii. verify that the compound is not available as a pharmaceutical grade product in the required formulation or concentration (if available in higher concentrations than needed, identification of the diluent is necessary and dilution with a pharmaceutical grade diluent is generally required), and
iii. justify use of the NPG product as an appropriate alternative.

2. Euthanasia Grade Sodium Pentobarbital

a. Euthanasia-grade sodium pentobarbital is a formulation that also contains phenytoin and/or other substances. Phenytoin sodium may produce cardiovascular collapse and/or central nervous system depression. It is a non-pharmaceutical grade compound and does not require scientific justification in the IACUC protocol **as a euthanasia agent.**

b. **Approved Uses of Euthanasia Grade Sodium Pentobarbital as an Anesthetic[2]**

- Perfusion under anesthesia
- Exsanguination under anesthesia

c. **Euthanasia grade pentobarbital may not be used for <u>any</u> survival anesthetic procedures.**

[1] Guide for the Care and Use of Animals, 2011, p. 81.

[2] "FDA approved euthanasia solutions may be used in those procedures in combination with the perfusion agent to perform perfusion and euthanasia as a single procedure"
OLAW Online Seminar, March 12, 2012. "Use of Non-Pharmaceutical-Grade Chemicals and Other Substances in Research with Animals"

3. 2,2,2 Tribromoethanol (TBE) (trade name: Avertin)

Pharmaceutical grade TBE is no longer available and investigators who wish to use this injectable anesthetic must make their own solutions with non-pharmaceutical grade TBE compounds. The side effects of TBE include acute inflammatory changes, local irritation, fibrous adhesions in the abdominal cavity, and death following one or repeated IP injections. Investigators must adhere to the following TBE guidelines:

- Strong scientific justification for the use of TBE must be included in the IACUC protocol.
- TBE degrades in the presence of heat and light, producing toxic by-products that are potent gastrointestinal irritants. Accordingly, great care must be taken to ensure that the product is made up fresh regularly, is sterile, and is stored properly (e.g., amber colored vials or plain glass wrapped in foil and stored in a cool, dark place).
- Even refrigerated and wrapped in foil, the material will degrade over time. Therefore, it is recommended that a new solution be prepared at least every 2 weeks.
- Discard the solution if:
    - the solution is less than pH 5
    - the solution develops an unusual discoloration (typically yellow) or forms a precipitate

4. Chemical Grade Compound Requirements

a. Substances formulated for injection must be prepared in a sterile manner. This requires sterile constituents (e.g., sterile powder, sterile diluents), a sterile container, and a means of keeping the preparation sterile. Injection vials are preferred as they make it easier to load a syringe and allow removal of solution without exposing the contents to air or contamination.

b. Use of vehicles will be evaluated on a case-by-case basis. **Use of some vehicles may limit amounts, concentration, and routes of administration.** Acceptable vehicles (sterile filtered if possible) include but are not limited to:
    - Distilled water
    - Phosphate buffered saline (PBS), (e.g., Hanks)
    - 60% (v/v) propane-1:2-diol (propylene glycol)
    - 0.5% (w/v) carboxymethyl cellulose
    - 10% (v/v) Tween 80 (polyoxyethylene (20) sorbitan mono-oleate)
    - 10% (v/v) ethyl alcohol
    - 50% (v/v) dimethylformamide
    - 50% (v/v) dimethyl sulfoxide (DMSO)
    - Cyclodextrins5 (e.g., 2-hydroxypropyl-beta-cyclodextrin, Trappsol®)

c. Containers (e.g., injection vial) must be labeled with the drug, concentration, date of preparation, and date of expiration.

d. When possible, prepared solutions must be passed through a syringe filter (0.22µm or finer) at the time of preparation. This can be done in the process of transfer to an injection vial. If there is any question about the sterility of a stored solution, it must also be filtered at the time of use. If filtering is not possible (e.g., nanoparticles), sterile components should be mixed using sterile technique.

e. Prepare only as much as can be used in a reasonable period of time. Drug solutions prepared and stored properly in a suitable injection vial can be kept for 1 month after which they must

be considered expired. Solutions must not be used if they are cloudy, discolored, precipitated or otherwise altered.

Two exceptions to this 1-month expiration timeline include:

- Ketamine and xylazine cocktails may be kept for up to 6 months
- Diluted buprenorphine HCl may be kept for up to 6 months and must be stored in glass vials as buprenorphine will lose efficacy over time when stored in plastic containers such as Eppendorf tubes, falcon tubes, and syringes. **Long-acting buprenorphine formulations (such as buprenorphine SR and Ethiqa XR) cannot be diluted for any reason due to the nature of the sustained-release formulation.**

f. pH of solutions must be between 4.5 and 8.0. Use of a solution with a pH outside this range must be addressed in the animal use protocol.

E. Expired Substances and Materials

1. **Rutgers University prohibits the use of expired drugs and reagents for euthanasia, anesthesia, and analgesia agents. Drugs cannot be used beyond their expiration date even if a procedure is terminal.**

2. When the expiration date provided is month and year, it is usable until the final day of the month listed.

3. For substances that are mixtures or have had alterations to the original pharmaceutical preparation, the **new, mixed substance expires in 30 days** (see V.D.4.e above for exceptions). If any component of the mixture expires in less than 30 days, use the original vendor expiration date.

4. **Expired materials** (ex. gauze, suture, etc.) can only be used in non-survival studies. See IACUC Policies: Non-USDA Species Survival Surgery and Non-Rodent Surgery/USDA Species.

5. All expired substances and materials must be clearly labeled 'EXPIRED' and stored separately from non-expired materials.

6. Expired substances and materials should be promptly discarded in accordance with Rutgers Environmental Health & Safety (REHS) policy. See CMR Controlled Substances Standard Operating Procedure for legal disposal of controlled substances.

7. Members of the IACUC and CMR perform announced or unannounced lab visits that include a review of the manner of storage, record keeping and for the presence of expired substances and materials. All expired substances and materials in animal study areas, including research laboratories, will be confiscated at the time of discovery without remuneration.

RUTGERS UNIVERSITY IACUC Document #E3	Use of Adjuvants
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 3/17/2021 (version 3.0)

I. Purpose

The purpose of this document is to provide guidance and resources for investigators that require adjuvant use in live animals.

II. Introduction

Adjuvants include any compound that enhances the immune response to an antigen. Adjuvants are commonly used for the *in vivo* production of polyclonal antibodies either to foreign or self antigens. Many adjuvants are commercially available, and selection is based on intended use and desired effect. Examples include vaccine development/use (low immune response), monoclonal/polyclonal antibody production and collection (moderate immune response), and induction of autoimmune disease (intense immune response). No adjuvant is ideal for all situations and all adjuvants produce varying undesirable side effects, including toxicity.

Commonly used adjuvants:

- Complete Freund's Adjuvant (CFA) – Water-in-oil emulsion containing heat-killed *Mycobacterium tuberculosis* and/or mycobacterial cell wall components; CFA induces a very strong inflammatory response at the injection site that can be painful to the animal. Repeated use can produce sterile abscesses, skin ulceration, and skin/tissue sloughing. CFA is typically only given for the initial immunization, followed by boosters of IFA.

- Incomplete Freund's Adjuvant (IFA) – Similar preparation to CFA, except IFA lacks the *Mycobacterium tuberculosis* component. Because IFA is less inflammatory, it can be safely used multiple times in the same animal.

- Other commercially available adjuvants include RIBI®, TiterMax®, Magic Mouse®, Specol®, montamides, Syntex Adjuvant Formulation (SAF), aluminum compounds, MF59, liposomes, etc.

III. Responsibilities

This document applies to any animal user at Rutgers University who is injecting adjuvant(s) into research animals.

IV. Methods

All adjuvants/antigens must be prepared using sterile technique. The preferred route of administration for most adjuvants is subcutaneous (SC).

Antigen/adjuvant injection site(s) should be disinfected. Shaving prior to disinfection is recommended. **CFA** should be the last resort regarding adjuvant choice; its use requires scientific justification along with

demonstration of a search for alternative adjuvants (databases such as ALTWEB or ALTBIB) for IACUC approval.

- animal protocols using CFA are automatically classified as pain Category E
- CFA is only allowed to be administered to each animal once (usually initial immunization)
- CFA should be prepared 1:1 (volume) with antigen
- if possible, prepare concentrations of CFA <0.1mg/ml (may not be possible for auto-immune disease induction)
- inject volume at multiple sites to minimize inflammation and avoid fusion of lesions if possible

Recommended volumes/sites for adjuvant antigen emulsion administration (all volumes in milliliters, ml):

	SC	ID	IP	FP	IM
Mouse	<0.1	*	<0.2**	<0.05**	<0.05***
Rat	<0.1	<0.05**	<0.5**	<0.1**	<0.1***
Rabbit	<0.25	<0.05**	*	*	<0.25***

SC = subcutaneous, ID = intradermal, IP = intraperitoneal, FP = foot pad, IM = intramuscular
*=not recommended **=requires justification ***=only one limb, requires justification

Post injection care – Post injection monitoring and care is required for all *in vivo* adjuvant use. The injection site should be monitored for at least three weeks (3 times per week) or until all lesions have healed. Lesions that ulcerate, necrose, or slough must be treated under the direction of the veterinary staff. Animals that show overt signs of pain (hunched appearance, poor coat, discharge around eyes, etc.) should receive analgesics (check with veterinary staff regarding choice).

Additional information can be found in the IACUC Policy Humane Endpoints Monoclonal Antibody Production.

RUTGERS UNIVERSITY IACUC Document #F1	Euthanasia of Research Animals **Date Issued:** 10/7/2015 (version 1.0) **Date Revised:** 2/17/2021 (version 3.0)

I. Purpose

This document provides investigators with acceptable methods of euthanasia most commonly used in research animals. All euthanasia methods are designed to minimize pain and distress prior to death.

II. Introduction

Methods of euthanasia used by investigators must be consistent with the most current version of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals and stated in an approved IACUC protocol. For information on additional methods of species-specific euthanasia not covered in this document, consult with the CMR veterinary staff.

III. Responsibilities

Any individual performing euthanasia of laboratory animals at Rutgers University must comply with the methods outlined in the approved protocol. Personnel performing euthanasia must be trained and proficient in the specific procedure(s) prior to working with animals.

IV. Procedures

A. Carbon Dioxide (CO_2)

CO_2 asphyxiation is an approved method of euthanasia for rodents and must be performed properly to be effective and humane. These procedures are practical only for rodents greater than 10 days of age. Neonatal rodents are very resistant to CO_2 euthanasia and prolonged exposure time is required in order to euthanize them (see A.13 below). The following procedures are designed to assure that CO_2 euthanasia is performed properly:

1. It is preferable that rodents be exposed to CO_2 in their home cage. If use of the home cage is not feasible place the animals in a secondary container that is easily sanitized. Use a euthanasia chamber where the animals can be seen without opening the chamber.
2. Rodents may be euthanized singly or in groups. Each animal must have space to stand on all four feet and have sufficient space to turn around on the cage floor. Rodents cannot be layered under any circumstances.
3. Mice from different cages should not be combined in one cage for euthanasia. Combining mice from different cages can be a stressor during euthanasia.
4. The gas flow rate must provide a balance between the time to unconsciousness and the adverseness of noise or high-velocity air movement from too-high flow rates. A displacement rate of 30% to 70% of the chamber volume/min is required.
5. Compressed gas tanks must be equipped with a pressure regulator and a flow meter.
6. Do not pre-fill the chamber with CO_2 prior to the addition of animals.
7. Empty the chamber of CO_2 gas before adding new animals.
8. CO_2 is heavier than air. Excess gas must be allowed to exit from the top of the chamber rather than from the sides or bottom to ensure that the animal is immersed in CO_2.
9. Euthanasia chambers must be kept clean and free of debris, urine and feces. Chambers and lids should be cleaned between each use.

10. Do not leave rodents undergoing CO_2 euthanasia unattended until death is assured.
11. Dry ice is not an acceptable means of producing CO_2 gas. The CO_2 source must be a compressed gas cylinder. 100% CO_2 is recommended.
12. CO_2 is not a practical method of euthanasia in neonatal rodents up to 9 days of age. A direct physical method such as decapitation is recommended. If CO_2 must be used to euthanize neonatal rodents, at least 50 minutes of exposure is recommended and a secondary method of euthanasia must be used.
13. Rodent fetuses are unconscious in utero and hypoxia does not evoke a response. Therefore, it is unnecessary to remove fetuses for euthanasia after the dam is euthanized using an acceptable method.
14. The use of CO_2 must be followed by verification of death by a secondary method, such as cervical dislocation, decapitation, thoracotomy, documented prolonged observation for at least ten minutes etc. unless the animals are euthanized using Euthanex systems.
15. Euthanex systems allow small animals such as mice and rats to remain in their home cage to reduce stress. The automated SMARTBOX CO_2 equipment has a timed cycle during which the system cannot be opened and provides efficient humane euthanasia in compliance with AVMA euthanasia guidelines. In addition, after the completion of the cycle the animals exhibit rigor when they are removed from the system. This confirms the death of the animal eliminating the need for doing another secondary method of euthanasia such as cervical dislocation.

B. Anesthetic overdose (inhalation)

1. Inhaled anesthetic overdose (e.g., isoflurane gas) can be used for the euthanasia of laboratory rodents and other species including birds. With inhaled anesthetic overdose, anesthetic concentrations can be rapidly achieved and maintained, but an increased concentration of inhaled anesthetic may be necessary to adequately euthanize an animal. With inhaled anesthetics, animals can be placed in a closed receptacle containing cotton or gauze saturated with an appropriate amount of concentrated liquid anesthetic, or anesthetic vapor can be introduced from a precision vaporizer. With precision vaporizers, oxygen is provided along with inhaled anesthetic gas, so time to death may be prolonged. When using the closed receptacle with volatile anesthetic, a barrier must be provided to ensure that animals will not come into direct contact with the anesthetic-soaked materials, as it is an irritant.

2. It is necessary to verify that death has been achieved with this method of euthanasia, either by performing a secondary physical method after the animal is confirmed to be non-responsive to toe pinch (e.g., decapitation, cervical dislocation, or thoracotomy) or by physical examination of cessation of respiration through prolonged observation. Prolonged observation requires that the animal be observed while actively exposed to the inhaled anesthetic for a minimum of 3 minutes after cessation of respiration, followed by an additional prolonged observation for at least ten minutes in room air.

3. Effective engineering controls should be in place to reduce exposure of researchers/technicians to volatile anesthetic vapors. In laboratory settings, personnel utilizing volatile anesthetics for euthanasia must do so within the confines of a ducted biosafety cabinet (Class II B2) or ducted chemical fume hood.

C. Injectable agents

1. **Barbiturates**
   Sodium pentobarbital is the most commonly used barbiturate. It is advisable to use a dose that is 3 times the recommended anesthetic dose for rodents. Sodium pentobarbital can be delivered IV or IP. Contact CMR Veterinarians for further recommendations for non-rodent species.

2. **Potassium Chloride**
   Potassium chloride may be used for euthanasia, but must be administered IV using a saturated solution and only when animals are non-responsive under general anesthesia. Contact CMR Veterinarians for further recommendations for use.

D. Tricaine Methanesulfonate (MS-222)

1. MS-222 is an anesthetic agent used in aquatic species, and is intended for the temporary immobilization of fish, amphibians, and other aquatic cold-blooded animals. At high doses, MS-222 is also commonly used for euthanasia of aquatic species.

2. An FDA-approved, pharmaceutical grade formulation of MS-222 must be used.

3. In laboratory settings, MS-222 must be prepared within a chemical fume hood and while wearing PPE as required by REHS.

4. MS-222 is acidic and should generally be used in a buffered solution. The most common buffered solution is composed of 10 g/L sodium bicarbonate in tank water to maintain a pH near 7.0. Buffered MS-222 is required for use with the most common species of laboratory fishes. For species that live in acidic habitats or have other requirements, please include a description of the preparation and use of MS-222 in the protocol.

5. MS222 in solution should be frozen or refrigerated in between use and should be disposed of appropriately if it discolors or is not effective. The expiration date on the label of the powder form should be followed.

6. Fish: Most fish older than 3 days post fertilization (dpf) can be euthanized via immersion in a minimum of 250-500mg/l MS-222 in buffered tank water. Fish must remain in solution at least 30 minutes following cessation of opercula movements. In larvae where there is no operculum, the heart can be visualized and death is confirmed when it stops beating. Due to species differences in response to MS-222, a secondary method of euthanasia is recommended in many fish to ensure death. For zebrafish, rapid cooling euthanasia is preferred to MS-222 immersion.

7. Aquatic frogs: Frogs are immersed in a minimum of 3g/l MS-222 in buffered tank water, although higher dosages of 5-10 g/l are recommended. Prolonged immersion (up to 1 hr) may be required. MS-222 euthanasia must be followed by a secondary method of euthanasia; usually decapitation that may be followed by pithing or double pithing. See below for more information.

E. Cervical Dislocation
Euthanasia <u>without a surgical plane of anesthesia</u> of mice by cervical dislocation is generally prohibited.

Cervical dislocation without anesthesia is only permitted when scientifically justified by the user in the protocol review form and approved by the IACUC.

All personnel performing cervical dislocation without anesthesia are required to demonstrate technical proficiency.

Decapitation is strongly recommended over cervical dislocation if a physical method without anesthesia is necessary to meet scientific aims.

F. Decapitation

Euthanasia without a surgical level of anesthesia of rodents, small rabbits, birds, amphibians, and fish by decapitation with a guillotine or, in the case of small animals, another cutting instrument such as scissors or scalpel blade is generally prohibited.

Decapitation without anesthesia is only permitted when scientifically justified by the user in the protocol review form and approved by the IACUC. For reptiles and amphibians, decapitation (whether under general anesthesia or not) must be followed by pithing unless otherwise justified and approved in the protocol.

All personnel performing decapitation without anesthesia are required to demonstrate technical proficiency. PIs are responsible for ensuring that the guillotine or other decapitating cutting device is in good operating condition and that the blade(s) are sharp and clean.

1. Place the animal's neck in the guillotine or other cutting device.

2. Ensure that the operator's digits are away from the animal's neck and then quickly close the cutting device to sever the neck, separating the animal's head from its body.

3. The guillotine or scissors must be cleaned thoroughly after use to keep blades clear of hair and tissue that may affect cutting ability. Decapitation equipment must be evaluated and sharpened regularly. A log of routine checks for sharpness and maintenance for each guillotine must be available for review during IACUC semiannual inspections. In the case where sending guillotines or blades for sharpening is not feasible, blades should be replaced annually.

4. CMR may be consulted for recommendations on blade sharpening.

G. Pithing

Following general anesthesia induction and primary form of euthanasia, pithing is generally required as a second-step method of euthanasia for most species of amphibians. Procedures other than those recommended below must be approved by the IACUC with scientific justification. Pithing requires dexterity and skill and should be performed only by trained personnel. Faculty should use judgment in deciding whether to perform pithing in the presence of students.

Pithing alone is generally not an acceptable primary method of euthanasia and should never be used for Xenopus (African Clawed Frogs) as it is difficult to bend the head forward to expose the atlanto-occipital space. Xenopus should be decapitated followed by pithing. Again, frogs generally must be fully anesthetized prior decapitation followed by pithing as a secondary form of euthanasia.

Recommended Procedures:

1. **Double pithing in frogs**
   a. Hold the frog facing away from your body, with the lower extremities extended.
   b. Grasp the frog with your first two fingers: first finger on the snout, second finger under the jaw.
   c. Flex the head forward (away from your body).
   d. Move probe down midline, over a bump (as a reference point) that is the occipital process until you come to the soft spot of the foramen magnum.
   e. Insert the probe quickly into the cranial vault and move the probe from side to side to destroy the brain.
   f. Turn the probe around into the vertebral canal and insert the probe into the full length of the vertebral canal to destroy the spinal cord.

2. **Decapitation and spinal pithing**
   a. Using heavy shears or a guillotine sever the spinal cord of the frog at the atlanto-occipital junction.
   b. Insert a probe into the cranial vault and move it from side to side to destroy the brain.
   c. Then insert it into the full length of the vertebral canal to destroy the spinal cord.

H. Rapid Cooling of Zebrafish (Danio rerio):

It is acceptable for zebrafish older than 3 dpf to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and opercula movements and subsequent holding times in ice-chilled water, specific to size and age. In larvae where there is no operculum, the heart can be visualized and death is confirmed when it stops beating. Fish must never come into direct contact with ice.

Zebrafish adults (approx 3.8 cm long) can be rapidly euthanized (10 to 20 seconds) by immersion in 2° to 4°C (36° to 39°F) water. Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 14 dpf for at least 20 minutes following loss of operculum movement.

Use of rapid chilling and use of buffered MS-222 alone have been shown to be unreliable euthanasia methods for zebrafish embryos < 3 dpf and should not be used, unless there is a secondary method such as the addition of household bleach solution to the culture system water at 1 part bleach to 5 parts water (i.e., minimum final concentration of sodium hypochlorite 1.5%).

I. Diluted Sodium or Calcium Hypochlorite Solution:

Immersion in diluted sodium or calcium hypochlorite solution at 500 mg/L. is acceptable for euthanasia of zebrafish embryos up to 7 days of age.

V. References

- AVMA Guidelines for the Euthanasia of Animals, current version
- Report of the ACLAM Task Force on Rodent Euthanasia, American College of Laboratory Animal Medicine, 2005
- Guide for the Care and Use of Laboratory Animals, current version

RUTGERS UNIVERSITY IACUC Document #F2	Humane Endpoints
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 2/17/2021 (version 3.0)

I. Purpose

This document covers the procedures to be followed by all Rutgers University animal users to establish humane endpoints for animals used in research, testing and teaching.

II. Introduction

This document provides guidance for determining appropriate humane endpoints for all research studies where animals may experience pain or distress. All personnel involved in animal research studies must strive to reach the scientific objective, balancing it with best humane methodologies. It is the obligation of the animal users to minimize or eliminate unnecessary pain and distress animals experience when used in research, research training, and biological testing activities. This obligation is clearly stated in the Animal Welfare Act Regulations, the Guide for Care and Use of Laboratory Animals, and other applicable local and national legislations.

The goal should be to use humane endpoints to minimize pain, distress, or suffering to the extent possible without compromising the scientific objectives of the experiment. The investigator also should determine experimental endpoints when the scientific information is obtained and the study can be terminated. Discussions with other scientists or veterinarians who have experience with the proposed model are also useful. Once potential adverse events are identified, all efforts should be made to minimize the ill effects, monitor and have proper endpoints established.

III. Responsibilities

All animal users are responsible for implementation and oversight of these procedures.

IV. Definitions

The **experimental endpoint** of a study is when the scientific aims and objectives have been reached.

The **humane endpoint** is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in unrelieved or severe animal pain and distress, including death. (the Guide, p27).

Stress versus Distress - The general public often uses “stress” and “distress” interchangeably, and frequently in conjunction with the term “suffering,” thus blurring distinctions between these concepts. Because there is in fact good scientific evidence for both an adaptive *stress* response and a state of *distress*, it is important to distinguish these terms.[3]

[3] Recognition and Alleviation of Distress in Laboratory Animals. National Research Council (US) Committee on Recognition and Alleviation of Distress in Laboratory Animals, . Washington (DC): National Academies Press (US); 2008.

The definitions of "stress" and "distress" as noted in The Guide for the Care and Use of Laboratory Animals (the Guide):

- **Stress** is a real or perceived perturbation to an organism's physiological homeostasis or psychological well-being. The transition of stress to distress depends on several factors. Of clear importance are stressor duration and intensity, either of which is likely to produce behavioral or physical signs of distress.
- "**Distress** may be defined as an aversive state in which an animal fails to cope or adjust to various stressors with which it is presented. But distress may not induce an immediate and observable pathologic or behavioral alteration, making it difficult to monitor and evaluate the animal's state when it is present. Both the duration and intensity of the animal's state are important considerations when trying to prioritize attention to and treatment of animal distress." (the *Guide*, p. 121).

Biomarker - The Food and Drug Administration (FDA) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic, pathologic processes, or pharmacologic responses to a therapeutic intervention."

V. Methods

A. Summary of selected criteria for euthanasia.
If any animal shows one or more of these clinical signs, they must be euthanized unless approved by the IACUC or a CMR veterinarian.
- Weight loss ≥10% within one week (considered rapid weight loss)
- Weight loss ≥ 20% over any time period
- Body condition scoring (BCS) <2
- Lesions (such as ulcerative dermatitis) covering ≥10% of the skin
- Rough hair coat, hunched posture, distended abdomen, or lethargy; especially if debilitating or prolonged (≥3 days)
- Diarrhea; especially if debilitating or prolonged (≥3 days)
- Coughing, rales, wheezing, or nasal discharge
- Distinct icterus (yellow skin) and/or anemia (pale skin)
- Rapid growth of mass (or masses), or clinical signs of neoplasia not related to study
- Central nervous system signs unrelated to experimental expectations, such as head tilt, tremors, spasticity, seizures, circling, or paralysis/paresis, especially if associated with anorexia
- Uncontrollable bleeding from any orifice
- Significant hypothermia (decrease in body temperature of $\geq 6^0$C from baseline)
- Markedly discolored urine, polyuria, or anuria
- Persistent, self-induced trauma
- Lesions interfering with eating or drinking
- Clinical signs of suspected infectious disease requiring necropsy for diagnosis
- Other clinical signs as judged by the Veterinary Staff to be indicative of moribund condition

B. To ensure appropriate endpoints are included in an animal study, the following should be established:
- Precise definition of the humane endpoint, including assessment criteria
- The frequency of animal observations
- Ensure all animal users responsible for making the animal observations and endpoint decisions are adequately trained to observe and recognize the intervention points
- State in the protocol the actions to be taken when the endpoint is reached

C. Possible endpoint actions could include:
   - Euthanize an animal to prevent pain or discomfort
   - Discontinue a painful procedure
   - Remove an animal from a study, thus relieving the pain or distress

D. Finding appropriate humane endpoints can be achieved by various methods or combinations of methods including those on the list below:
   - Literature review
   - Previous work with the animal model, experimental compound or class of compounds
   - Pilot studies
   - New applicable technology (biomarkers, imaging, early indicators)
   - Expert consultants (Veterinarians, Toxicologists, Scientists)
   - Discussions between the animal users and CMR veterinarians and/or the IACUC to understand scientific objectives and limitations, and help in developing a stronger scientific and humane protocol

E. Studies that achieve their scientific objective prior to their experimental endpoint should be terminated earlier.

F. The relevant humane endpoints must be described in the protocol. All animal users must be informed and trained in their roles in the study.

G. **Parameters to consider when formulating humane endpoints include:**

1. Changes in external physical appearance or other clinical signs

Examples include: fur/coat, posture, gait, body condition, swellings or masses, prolapse, sunken eyes, or head tilt.

For endpoints in tumor studies ,refer to the IACUC Policy Cancer Studies / Tumor Models for more details.
Animals should be examined regularly and documented by appropriately trained animal users. The frequency of such examinations will depend on the species, whether any previous abnormalities have been observed, timing and nature of the anticipated effects, and the objectives of the study. Frequency of examinations should increase as animals approach the anticipated humane endpoint. Once an endpoint is reached the pre-planned action should occur. A chart of commonly observed clinical signs and conditions per species is provided below.

2. Physiological changes

Examples include: body temperature, weight loss, heart rate, respiratory rate

Information regarding various physiological changes should be monitored to assist in the determination of endpoints. The relevance of these parameters should be established. Two common physiological changes that have been used are body weight and body temperature.

Significant body weight loss may be one of the most sensitive indicators that an animal's condition is deteriorating, particularly if it occurs over a short period of time. Body weight loss should be compared to age/sex matched controls. In young animals that have not reached their adult body weight, an abnormal condition may be indicated by a reduced rate of weight gain when compared to

the appropriately matched control animal, rather than an actual weight loss. If body weight is used as an endpoint, the protocol should define the frequency of weighing, the individuals responsible for performing weighing, and analysis of the weighing. For studies where weight loss greater than 20% is expected and is still consistent with good animal welfare, justification for the necessity to allow this must be included in the protocol.

Indications for euthanasia (weight loss):

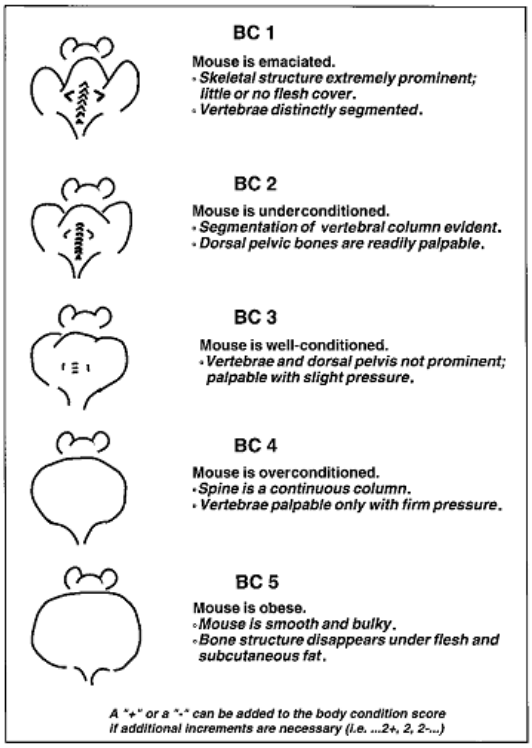
- Weight loss ≥10% within one week (7d) that cannot be corrected by fluid therapy and is due to loss of lean body mass
- Overall weight loss ≥20%
- BCS < 2

Note: BCS should be used when body weight does not accurately reflect the mouse's condition (such as tumors, pregnancy, ascites, juvenile rodents ≤ 50 days).

Body condition scoring (BC/BCS) can be used instead of weighing, especially if there are confounding factors such as tumor models, pregnancy, ascites production, or young growing animals.

The scorer picks up the mouse at the base of the tail and passes a finger over the sacroiliac bones (dorsal pelvis). Body condition is typically scored on a scale of 1-5, as described below:

1. Muscle wasting is advanced, fat deposits are absent, and bones are very prominent.
2. Bones are prominent. This suggests the mouse is becoming thin and its health is declining. Further decline of condition would warrant euthanasia.
3. Bones are palpable but not prominent. **This is the optimal condition**.
4. The mouse is well fleshed and bones are barely felt.
5. The mouse is obese and bones cannot be felt.



Changes in body temperature for certain types of studies may be an endpoint determinant. Hyperthermia or hypothermia has been successfully used in infectious studies in numerous published studies.

3. Diagnostic laboratory testing

Examples of biochemical parameters include: Blood, urine, tissues, and cerebral spinal fluid can be used to analyze hematological and bio markers.

Various hematological, clinical chemistry and urinary parameters can provide an indication of an animal's condition. Consideration should be given to collecting and monitoring parameters that may be useful in assessing an animal's well-being. In determining toxicity to certain organs such as liver and kidneys, serum chemistry has been a valuable tool.

4. Behavioral signs

Observing changes in behavior can be used to assess changes in the well-being of an animal. Behavioral changes that contribute to analysis for endpoints can be considered in 4 general categories:

- Response to external stimuli - e.g., response to touch
- Occurrence or changes in the frequency of behaviors that might occur as a result of pain or discomfort - e.g., vocalization, licking, biting, or guarding
- Adverse behaviors relevant to the model - e.g., lameness for arthritis studies
- Non-specific behaviors – e.g., sleeping patterns, eating and drinking patterns, and grooming

For effective behavior analysis it is critical that the monitoring personnel are trained to know normal and abnormal behavior for the species and even individual strains. Pain and distress monitoring often involves behavior observation and/or scoring. Pain and distress scoring is a method to convert subjective animal observations into an objective scoring system which can be helpful in assessing animal behavior. (see table below)

5. Severe pathology

Moribundity

A moribund animal is one that is near death and may be comatose or unresponsive to stimuli, exhibit dyspnea, hypothermia, prostration, etc. Although the goal of a sensitive humane endpoint is to intercede before a moribund state occurs, euthanasia is indicated if it does occur.

Mortality

Death as an endpoint should be avoided and **must** be justified to the IACUC. Justification must include how it was determined no alternatives exist, what will be gained by allowing the animal to die, rationale for withholding treatment for clinical signs, and the expected mortality rate. Death as an endpoint requires a very specific monitoring plan that details monitoring parameters and documentation of observations.

Score sheets

Endpoints are sometimes determined by using a list of key signs, and behavioral observations to evaluate the extent of deviation from normal. The key signs and observations are listed on score sheets or checklists. These are helpful in ensuring that appropriate observations are made, consistently interpreted, and properly documented. Signs and observations are to be recorded as

present (+) or absent (-) or a degree (0 – 3) representing normal to severe. By convention, negative signs indicate normality. A cumulative rating may be obtained by adding the score for each category. An increase may indicate deviation from normal. This can be interpreted as an indication of increasing pain and distress or identify a threshold which would indicate an intervention or endpoint. Score sheets may need to be specific for each experimental procedure, each species and even each strain.

Establishing a Plan

- A plan is required to follow animal care and monitoring procedures.
- The plan must identify personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and intervention.
- The plan includes score sheets or checklists and intervention as designated in the protocol.

Record Keeping

A laboratory notebook, animal medical records or another officially designated record system must be used to document the monitoring events, monitoring data and actions when the endpoint point is reached. Records should be readily available for veterinary staff to review.

6. Guidelines for Some of the Potential Signs Associated with Pain or Distress in Rodents and Rabbits[+]

Clinical Signs	Mice	Rats	Guinea pig	Hamsters, Gerbils	Rabbits
Decreased Food and/or Water Consumption	X	X	X	X	X
Weight loss	X	X	X	X	X
Self-imposed isolation/hiding	X	X	X	X	X
Self-mutilation, gnawing at limbs	X	X	X	X	X
Abnormal Breathing (rapid or labored)	X	X	X	X	X
Grinding Teeth		X	X	X	X
Biting /Aggression (strain variation)		X		X	X
Unkempt Appearance (Erected, Matted, or Dull Hair coat)	X	X	X	X	X
Abnormal Posture/Positioning/movements (e.g., head-pressing, Hunched Back, Staggering)	X	X	X	X	X
Tearing (including Porphyria+), Lack of Blinking Reflex, palpebral ptosis	X	X[+]	X	X	X
Dilated Pupils			X		X
Prolapse of third eyelid					X
Muscle Rigidity, Lack of Muscle Tone	X	X	X	X	X
Dehydration/Skin Tenting/Sunken Eyes	X	X	X	X	X
Twitching, trembling, tremor	X	X	X	X	X
Abnormal Vocalization (Rare)	X	X	X	X	X
Back-arching (cat stretch), writhing		X			

Clinical signs can be used for assessing morbidity in non-rodent (NHPs, dogs, pigs, ferrets) species:

(1) Decreased Appetite
(2) Weight Loss >20% (e.g., failure to gain weight compared to age matched controls)
(3) Abnormal Heart Rate (increased/decreased)
(4) Changes to peripheral pulses (bounding or weak, thready), or blood pressure
(5) Abnormal Breathing (rapid, shallow or labored slow)
(6) Dehydration (skin turgor, mucous membranes, urinary output)
(7) Body Temperature (increased, decreased)
(8) Changes in musculoskeletal/neurologic function (twitching, tremors, seizures, convulsions, paresis, hyperesthesia, decreased reflexes, lameness)
(9) Vocalization at handling
(10) Mucous membrane discoloration
(11) Self-mutilation, i.e., autotomy, autophagia
(12) Depression, lethargy

For species not identified please consult a veterinarian to define specific guidelines.

RUTGERS UNIVERSITY IACUC Document #F3	Humane Endpoints: Monoclonal Antibody Production in Rodents
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 5/19/2021 (version 3.0)

I. Purpose

The purpose of this policy is to provide direction and reference material regarding the humane endpoints of production of monoclonal antibodies (MAb) in rodents. For more information on monoclonal antibody production in other species, please contact CMR.

II. Introduction

MAb production results in accumulation of ascites fluid and is likely to cause pain and distress. The NIH concurs there is scientific necessity for this method. However, tissue-culture methods for the production of monoclonal antibodies should be adopted as the routine method unless there is a scientifically justified reason why they cannot be used. Accordingly, IACUCs are expected to critically evaluate proposed uses of the rodent ascites method by investigators.

III. Responsibilities

Animal users who are involved in the production of MAb in rodents are responsible for following this policy.

IV. Methods

A. In vitro methods - *In vitro* methods must be considered first. Refer to the below list for some potential commercial sources for in vitro production of monoclonal antibodies.

1. Covance Research Products: http://covance.com
2. Taconic Biotechnology: http://www.taconic.com
3. Cell Essentials, Inc: http://www.cell-essentials.com

B. In vivo antibody production

1. **Immunization Procedure** - Less toxic, alternative adjuvants to Complete Freund's Adjuvant (CFA) should be used; examples include Magic Mouse, TiterMax Gold, RIBI, and Aluminum salts. The use of CFA requires scientific justification. CFA/antigen mixtures should be limited to primary immunization and Incomplete Freund's Adjuvant (IFA) should be used in subsequent booster inoculations. Refer to IACUC Policy Fluid Administration and Collection in Rodents regarding proper needle size and injection volumes.

2. **Priming Agents**- Priming agents to promote ascites are generally administered IP prior to inoculation of hybridoma cells. Priming of the peritoneal cavity is often accomplished through an IP injection of ≤0.20 ml pristane.

3. **Induction of Hybridoma Cells**– Rodent-derived hybridomas must be tested for the presence of adventitious viral and mycoplasma agents prior to inoculation into mice in order to prevent potential transmission of murine infectious agents into animal facility experimental colonies. Refer to IACUC Policy Evaluation of Cell Lines and Rodent-Derived Biologicals.

4. **Ascites** - The cranial displacement of the diaphragm due to ascites is associated with rapid and labored breathing. There is a limit of 3 abdominal taps per animal (two taps in live animals and a final tap after euthanasia). General anesthesia is recommended during tapping. 1-2ml of warm (~37°C) 0.9% physiologic saline should be administered subcutaneously to help prevent shock post tap. Body weight of mice should not exceed 20% of the normal weight of age- and sex-matched animals of the same strain from the onset of ascites.

5. **Clinical Signs and Humane Endpoints**- Animals must be observed for signs of distress and pain. Animals that show signs of excessive distress or appear debilitated after any of the taps should be given fluids or euthanized. Animals must be euthanized when the following prolonged symptoms are observed: inappetence, inactivity, diarrhea/constipation, hunched posture, or rough coat. Acute symptoms that require euthanasia are hypothermia, tachypnea, labored breathing, pallor, inability to remain upright, or any other clinical signs indicated in IACUC Policy Humane Endpoints or CMR Veterinary Staff recommendation.

6. **Frequency of Observation–** Animals must be evaluated every other day during the first post-inoculation week by the lab. However, once ascites fluid accumulation and peritoneal cavity distention is noted, daily observation (including weekends and holidays) of animals is required.

Summary of Ascites Production

Fluid volume, site of injections, needle sizes	IACUC Policy Substance Administration and Blood Collection in Rodents
Testing cell lines for murine viruses	IACUC Policy Evaluation of Cell Lines and Rodent-Derived Biologicals
Priming	≤0.20 ml pristane
Number of taps	Maximum of two in a live animal (3rd after euthanasia)
Fluid volume administered	IACUC Policy Substance Administration and Blood Collection in Rodents
Monitoring after hybridoma inoculation	3 times a week during the first week, then daily
CFA use	Need scientific justification (typically only one CFA injection per animal), IACUC Policy Use of Adjuvants
Fluid replacement after ascites harvesting	1-2 ml warm saline SC
General anesthesia during tap	Recommended to prevent pain and distress
Humane endpoints	IACUC Policy Humane Endpoints

V. References

- The 1999 report of the National Research Council Monoclonal Antibody Production http://grants.nih.gov/grants/policy/antibodies.pdf
- OLAW: http://grants.nih.gov/grants/olaw/references/dc98-01.htm

RUTGERS UNIVERSITY IACUC Document #G1	Overcrowding and Single Housing for Rodents
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 2/16/2022 (version 4.0)

I. Purpose

This document establishes guidelines for single and group housing of adult and juvenile rodents.

II. Introduction

AAALAC Position Statement:
"AAALAC International expects accredited institutions to comply with all national or regional regulations, policies and guidelines, as well as conditions of funding. Additionally, AAALAC International considers performance standards paramount when evaluating the space made available in cages or pens for housing animals used for research, testing or teaching. The performance criteria described in the ILAR *Guide*, *Ag Guide* and ETS 123 are used by AAALAC in assessing the adequacy of cage or pen space available to the animal(s)."

III. Responsibilities

All animal users at Rutgers University must comply with this document.

A. Overcrowded Cages

1. PIs are responsible to ensure that cages do not become overcrowded by proactively separating animals appropriately and by not combining animals that would exceed permitted cage density in any situation, including for euthanasia.

2. If CMR staff identify overcrowded cages, the cages will be marked with an "Overcrowded Cage" notification card. The card is dated and the PI or designee is notified. In cases of chronic overcrowding, the PI may be billed an overcrowded cage notification fee for every cage that is identified as overcrowded.

3. If the PI does not correct overcrowded cages within 72 hours (including weekends and holidays), CMR staff will separate the animals into the appropriate number of cages (e.g., 15 newly weaned mice divided into 3-4 cages by sex). The PI will be billed a technical services fee for each cage separated by CMR, as necessary to comply with this policy.

4. Severely overcrowded cages (e.g., 15 or more mice, cages with litters of different ages) must be separated as soon as possible within 24 hours of notification. CMR may break up severely overcrowded cages without prior PI notification in the interest of animal welfare. The PI will be billed a technical services fee for each cage separated by CMR, as necessary to comply with this policy.

5. Excessive numbers of overcrowded cages or continued disregard for this policy may be reported at the discretion of CMR staff to the IACUC.

B. Single Housing of Social Animals

1. The PI is responsible for justification for single-housed social species. "Single housing of social species should be the exception and justified based on experimental requirements or veterinary related concerns about animal well-being. In these cases, it should be limited to the minimum period necessary, and where possible, visual, auditory, olfactory, and tactile contact with compatible conspecifics should be provided.[4]"

2. The PI is responsible for identification of the reason for each singly housed cage on each cage card using CMR's standardized method. Single housing, including the reason for single housing, will be identified on the cage.

3. All singly housed rodents require additional environmental enrichment unless justified in the protocol: See IACUC Policy Enrichment and Social Housing.

IV. Definitions

The cage- and species- specific requirements are based on the recommended minimum space for commonly used laboratory rodents housed in groups as described in the *Guide* 8th ed., page 57, table 3.2. **For specific caging sizes in each vivarium, consult the supervisor in your specific facility**.

Animals	Weight (g)	Floor Area/ Animal,[a] in^2 (cm^2)	Height,[b] in (cm)	Comments
Mice in Groups [c]	<10	6 (38.7)	5 (12.7)	Larger animals may require more space to meet the performance standards.
	Up to 15	8 (51.6)	5 (12.7)	
	Up to 25	12 (77.4)	5 (12.7)	
	>25	≥15 (≥96.7)	5 (12.7)	
Female + Litter (mouse)		51 (330) (recommended space for the housing group)	5 (12.7)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d]
Rats in Groups [c]	<100	17 (109.6)	7 (17.8)	Larger animals may require more space to meet the performance standards.
	Up to 200	23 (148.35)	7 (17.8)	
	Up to 300	29 (187.05)	7 (17.8)	
	Up to 400	40 (258.0)	7 (17.8)	
	Up to 500	60 (387.0)	7 (17.8)	
	>500	≥70 (≥451.5)	7 (17.8)	
Female + Litter (rats)		124 (800.0) (recommended space for the housing group)	7 (17.8)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d]

[a] Singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.
[b] From cage floor to cage top.

[4] The Guide for the Care and Use of Laboratory Animals, 8th ed., NRC Press, 2011.

[c] Consideration should be given to the growth characteristics of the stock or strain as well as the sex of the animal. Weight gain may be sufficiently rapid that it may be preferable to provide greater space in anticipation of the animal's future size. In addition, juvenile rodents are highly active and show increased play behavior.

[d] Other considerations may include culling of litters or separation of litters from the breeding group, as well as other methods of more intensive management of available space to allow for the safety and well-being of the breeding group. Sufficient space should be allocated for mothers with litters to allow the pups to develop weaning without detrimental effects for the mother or the litter.

V. Methods

A. Non-Breeding Rodent Summary

Refer to the above table for specifics regarding maximum number of rodents in a particular size cage. This includes animals awaiting euthanasia.

B. Breeding Rodent Summary

1. Monogamous pairing: 1 resident male and 1 female. This method is preferred to prevent overcrowding provided litters are weaned at 21 days of age. Monogamous pairing takes advantage of the post-partum estrus that results in more efficient reproduction and shorter time between litters. Pups must be weaned at 21 days so that newborn pups will not be compromised, unless delayed weaning (up to 28 days) is specified in the IACUC protocol or with CMR veterinary consultation.

2. Trio breeding: 1 resident male and 2 females. Trio breeding cages must be closely monitored so that litters can be promptly weaned to avoid overcrowding and trampling of pups. The pregnant females **must** be separated prior to parturition. Trio breeding must be approved in the IACUC protocol. If trio breeding cages are not properly managed by the PI and repeated overcrowding is observed, the PI may be prohibited from using this breeding scheme.

3. Harem breeding: 1 resident male and more than 2 females. Harem breeding cages must be closely monitored so that litters can be promptly weaned to avoid overcrowding and trampling of pups. The pregnant females **must** be separated prior to parturition. **Harem breeding must be scientifically justified in the IACUC protocol.** If harem breeding cages are not properly managed by the PI and repeated overcrowding is observed, the PI may be prohibited from using this breeding scheme.

RUTGERS UNIVERSITY IACUC Document #G2	Satellite Animal Facilities
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 3/17/2021(version 3.0)

I. Purpose

Under special circumstances it may be appropriate to house animals outside of centralized animal care facilities. This document establishes guidelines to be met by investigators so that the IACUC can assure that animals receive proper care and that the animals do not pose a hazard or inconvenience to people in the building in which they are kept.

II. Introduction

"Animals should be housed in facilities dedicated to or assigned for that purpose, not in laboratories merely for convenience. If animals must be maintained in a laboratory to satisfy the scientific aims of a protocol, that space should be appropriate to house and care for the animals and its use limited to the period during which it is required." p 134, the Guide.

"All animals should be observed for signs of illness, injury or abnormal behavior by a person trained to recognize such signs. As a rule, such observations should occur at least daily, but more frequent observations may be required, such as during post-operative recovery, when animals are ill or have a physical deficit, or when animals are approaching a study endpoint." p 112, the Guide.

The environment within an animal facility must provide for the health, safety, security, comfort, and well-being of the animals and staff, regardless of location.

III. Responsibilities

This document applies to all animal users at Rutgers University who house their animals outside of CMR vivaria.

IV. Definitions

A **satellite animal facility** is defined as an animal holding area outside of a vivarium for more than 12 hours (USDA species) or 24hrs (non-USDA species including mice and rats).

V. Methods

A. Indications for Satellite Facility - Use of a satellite animal facility will be allowed only when there is either 1) insufficient animal holding space within CMR facilities or 2) there is a demonstrated need to house animals immediately adjacent to research laboratories for scientific purposes when research space near CMR facilities is not available. Convenience of proximity is not considered a demonstrated need. Housing of animals outside of CMR animal facilities must be approved in an IACUC protocol.

Examples of instances in which satellite housing is appropriate include:

- Species which require specialized housing not generally available in animal facilities (e.g., fish)
- Projects that require the use of expensive specialized equipment which is located in a lab and must be in close proximity to the animals

B. Planning and Construction of a Satellite Facility

The IACUC will review the plans and needs for a satellite animal facility and render final approval. A CMR veterinarian and the IACUC must be involved early in the evaluation, design, building, or renovation of a satellite animal facility. Animals, animal odors, and allergens must not adversely affect people in the building.

The use of a satellite facility must be part of an approved IACUC protocol; an initial facility inspection by the IACUC is required before approval and relocation of animals. CMR personnel can provide animal care and management of a satellite animal facility on a per diem recharge system, if needed. Because of increased efforts and costs involved in servicing a satellite animal facility, either increased per diem rates or specific cost recovery charges may be negotiated between the PI and CMR.

C. Satellite Requirements

1. All satellite facilities must have functional and support spaces required for optimum animal care and use; refer to Appendix I for environmental and monitoring requirements for rodents. Veterinary staff must have 24-hour access to the satellite facility at all times. If surgery is planned, appropriate space and equipment must be provided.

2. All satellite facilities must maintain the same environmental standards as found in the central CMR vivaria for the given species. Research staff must report any deviations outside the acceptable range (rodent ranges can be found in Appendix 1; a CMR clinical veterinarian or facility supervisor can provide acceptable ranges for other species). Additionally, all sick animals and animal deaths must be reported. Reports must be made via phone call, text message, and/or email within 24hrs of detection to CMR management and/or veterinary staff.

3. All animals must be observed at least once daily including weekends and holidays. Observations must be documented each day and documentation must be available for review by CMR staff or the IACUC at any time.

4. The animal program conducted in a satellite animal facility will be overseen by the IACUC and must adhere to IACUC policies and review procedures, including semi-annual inspections.

5. Regardless of whether a satellite facility is part of the university (if the animals are owned by the University) or if the facility is shared by both university and non-university users, it must be part of the AAALAC accreditation program. It will be the responsibility of the satellite users to maintain the facility in an accreditable condition.

6. Adequate security measures for the facility must be developed and implemented in conjunction with Rutgers security personnel.

7. Each satellite facility, in consultation with CMR, must develop an appropriate emergency preparedness plan and the plan must be available in the satellite facility.

D. Termination of a Satellite Facility

1. Use of a satellite animal facility is subject to immediate termination by the IACUC and/or the Attending Veterinarian or designee if this policy is not followed and/or in the case of significant animal welfare concerns.

2. The termination of the use of a satellite facility must be submitted in writing to the IACUC by the PI. The IACUC will notify regulatory agencies accordingly.

VI. References

- The Guide for the Care and Use of Laboratory Animals, 8th ed, NRC Press, 2011
- Animal Welfare Act

Appendix I - Environmental and health monitoring of rodents housed in a satellite facility

	Condition	Description
1	Light	dark/light 12/12 automatic timer
2	Temperature	accepted range 18-25°C (68-79°F)
3	Humidity	between 30-70%
4	Feed / bins	Sanitized, vermin-proof containers
5	Water	potable water
6	Room Air	minimum 10-15 fresh-air exchanges/hour, keep animal room doors closed
7	Enrichment	Social housing, enrichment objects in each cage
8	Animal manipulation	performed under Biosafety cabinet for allergy prevention and biosecurity, when possible and as appropriate
9	Vermin control	log of daily trap monitoring, where humane rodent traps are deployed
10	Cage changing	static cages weekly, ventilated cages bi-weekly or more as needed
11	Animal monitoring	daily (including weekend and holidays) or more as indicated in the IACUC protocol, daily log must be kept and available at all times
12	Biohazard	follow REHS recommendations
13	Disaster plan	portable heater, cooling, back-up power, feed/water provisions
14	IACUC Policies	Must follow all applicable documents - e.g., Enrichment and Social Housing, Overcrowding and Single Housing for Rodents, Humane Endpoints, Food and Fluid Restriction / Regulation

Note – Individual Rutgers campuses and facilities may have additional requirements for satellite facilities based on location

RUTGERS UNIVERSITY IACUC Document #G3	Transportation of Rodents Within Rutgers University
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 4/21/2021 (version 3.0)

I. Purpose

This document covers the procedures to be used by animal users when transporting research animals within Rutgers.

Animal transfers to other institutions is detailed via Import/Export standard operating procedures (SOPs). Animals cannot be transferred to or from another institution without prior approval/authorization from both institutions. Animal transfers outside the Rutgers system must be performed via a CMR approved third-party courier service and are not included in this document.

II. Introduction

It is critical to ensure safe and appropriate transportation of rodents when animals are taken through general use areas. Rodents should be transferred in a manner that:

- Prevents escape in the vehicle, including in the event of an accident (within reason)
- It is not readily apparent that animals are being transported
- Minimizes spilling or aerosolization of bedding and animal allergens outside the animal facility; only clean containers or cages should be used to transport animals outside animal facilities
- Animals or caging containing hazardous materials must be in secondary containment or appropriate transport crates. All Rutgers Environmental Health & Safety (REHS) directives must be followed in moving and handling cages and animals.
- CMR recommends an acclimation period of at least 3 days for transfers between campuses/facilities or to satellite facilities if animals are not used immediately for terminal procedures.

III. Responsibilities

PIs or designees are responsible for implementation and oversight of these procedures. Food and water must be provided to any animals being transported.

IV. Definitions

Transport/shipping containers are specially designed to minimize shipping stress and exposure to infectious agents. Most are reusable, and can be autoclaved before each use. At a minimum the container should: have a secure lid; allow for airflow through openings fitted with HEPA filters on at least two sides and on top; the ability to convert the container to either one or more interior compartments; ideally a clear lid for easy inspection during transit (not always available); and be made of plastic.

All containers being used in vehicular transport over 20 miles must meet International Air Transport Association (IATA) specifications.

V. Methods

A. Identification – All boxes containing live animals that are being shipped/transferred must be marked with the following information: Rutgers PI last name, Rutgers IACUC protocol number, numbers of animals in box (preferably broken down by sex), along with recipient information including PI, protocol number, emergency contact information, and address (when being transferred to different PI/protocol).

B. Intra-Rutgers Transfer – Laboratory staff are not permitted to transport animals between Rutgers campuses, between Rutgers animal facilities on a single campus, or between suites/rooms within a Rutgers animal facility unless pre-approved and authorized by CMR. This may include transport by non-CMR staff in a private vehicle. CMR staff handles most animal transfers. To request transfer of animals, contact the Area Supervisor of the home facility (where the animals were originally housed) and complete the necessary form(s). This does not apply to moving animals from a colony room to a designated procedure room.

The receiving protocol must include the strain(s) and sufficient animal numbers prior to transport of animals if animals are being transferred to a different protocol as part of the move.

Quarantine may be required if animals are transported to a facility of higher biosecurity. In some cases animal transfers may be denied at the discretion of the CMR Director or their designee.

C. Transfer between Racks within a Room – Movement of cages between racks in a single room is generally discouraged. Consult with CMR staff before moving cages. **If the rack contains sentinel animals, under no circumstances are these cages to be moved unless approved by CMR Director or their designee.**

D. Transfer from Animal Facility to Investigator Laboratory

1. Laboratory animals may only be transported in their primary cage or an approved transport device that is escape-proof and adequate in size. Secondary containment may be required for some transport containers.

2. Animals must be moved out of the animal facility in a procedure prescribed by the CMR. Animals must be moved between floors using a freight elevator only (where available). Contact Area Supervisor for details.

3. Care must be taken to minimize the time spent in common hallways or lobbies when transporting animals between animal facilities and laboratories. Whenever possible service elevators and corridors should be used**. Cages and carts must be covered to ensure animals are shielded from public view.** Make sure the cover does not obstruct air flow to the cage(s).

4. Turn water bottles around or remove water bottles so that water does not spill into the cage during transport. **Be sure to turn the bottle around on arrival at the lab to enable animals to access water.** Gel water may be used as an alternative.

5. Efforts must be made to minimize the amount of stress animals may experience during transport.

6. On arrival at the lab, place cages in a designated secure area where other people working in the room have minimal exposure to potential allergens, if possible. The preferred location is a certified fume hood; however, no chemicals or other materials are permitted in the hood while animals are present.

7. Food and water must be provided to animals in PI labs at all times, unless specified in the IACUC protocol.

8. Return soiled cages to the animal facility promptly; do not dump bedding in the laboratory. Bag used cages after removal of animals. Follow animal facility procedures for returning used cages.

9. All REHS recommendations regarding hazardous animals, bedding, and cages must be followed in the lab. Refer to IACUC protocol and/or REHS for details.

10. When cages of animals are transported on a cart, the cages must be secured (e.g. straps, anti-slip mats, bungee cords).

11. In the event an animal escapes during transport, CMR must be notified immediately.

Note: **Many facilities at Rutgers University do not permit the return of animals to the animal facility once they have been taken to the PI's lab.** Contact the Area Supervisor to determine if animals are permitted to exit and return to a specific animal facility prior to removal of animals.

E. Use of Vehicles for Animal Transportation

1. All vehicular intra-Rutgers transport of rodents must be in a CMR approved shipping crate and provided with fresh bedding, food, and gel water. Secondary containment may be required at the discretion of the veterinary staff.
   a. At the discretion of CMR, a standard rodent cage may be used; in this case the water bottle must be removed and gel water placed on the cage floor and the lid must be secured to ensure it does not separate from the cage during transport.

2. Animal crates must be covered during transport. Make sure the cover does not obstruct air flow to the cage(s).

3. Animal cages must be kept flat on the floor of vehicle and cannot be tilted or laid on their side. When possible, cages should be secured to preventing shifting or falling over.

4. Throughout transportation the ambient temperature in the vehicle may not exceed 79°F nor fall below 68°F and comply with species-specific requirements. The ideal temperature for most rodent species is 70°F. Vehicles that cannot achieve these parameters cannot be used to transport live animals.

5. Transportation of USDA regulated rodent species must be in accordance with standards as described in the Animal Welfare Act.

6. Animals must be picked up and delivered at designated loading docks only, when present. Animals should not be carried on paths, sidewalks, or streets during delivery, if it can be avoided.

7. An adequate attempt must be made to prevent exposure of the driver to animal allergens and potential zoonotic diseases. This includes proper crating of animals, but in some cases may require additional measures such as use of respirators.

8. Vehicles used for animal transport must be sanitized after animals have been transported. At a minimum this requires wiping down of surfaces with a CMR accepted agent such as MB-10 or clidox. Any applicable CMR SOPs must be followed.

9. Animals being transported must not be left unattended, unless in an emergency situation.

10. In the event of a vehicle break-down during animal transport, CMR should be contacted as soon as possible to arrange delivery or return the animals to the home facility.

11. Smoking, eating, or drinking are not permitted in the vehicle when animals are being transported.

12. Disinfectant (e.g. MB-10, Clidox) along with basic PPE (gloves, gowns), should be available in vehicles transporting live animals.

13. University-Owned Vehicles: Driver must have prior approval by Rutgers University to operate a university-owned vehicle.
    a. University-owned vehicles used for animal transport must be inspected as a part of each semi-annual inspection to ensure proper functioning of all systems, especially those related to safety and climate control.

14. Privately-Owned Vehicles: Privately-owned vehicles must meet all criteria outlined in this document plus that outlined below.
    a. Privately-owned vehicles can only be used if a University-owned vehicle is not available or is approved by a CMR veterinarian or supervisor. The vehicle must have adequate climate control to maintain the temperature within the accepted range of 68°F-79°F and comply with species-specific requirements at all times.
    b. The CMR Director or designee must pre-approve the use of a private vehicle and driver (if non-CMR staff) for animal transport; this includes inspection of the vehicle prior to transport.
    c. Animals must be transported in the passenger cabin of the vehicle.
    d. Plastic sheeting (or similar material) should be placed on the vehicle floor and the animal cages placed on top. The sheeting must be discarded after transport is complete.
    e. No stops are allowed except in emergencies once the transport has begun.
    f. Private vehicles cannot be used to transport hazardous animals/cages unless pre-approved by CMR and REHS.

15. Animals are not permitted to be transported via public transportation, university shuttle, motorcycle, moped/scooter, or bicycle.

VI. References

- Animal Welfare Act
- The Guide for the Care and Use of Laboratory Animals, current edition

RUTGERS UNIVERSITY IACUC Document #G4	Daily Observation of Animals **Date Issued:** 10/7/2015 (version 1.0) **Date Revised:** 4/21/2021 (version 3.0)

I. Purpose

This policy establishes guidelines for daily observation of all animals maintained at Rutgers University.

II. Introduction

"All animals should be observed for signs of illness, injury, or abnormal behavior by a person trained to recognize such signs. As a rule, such observation should occur at least daily, but more frequent observations may be required, such as during postoperative recovery, when animals are ill or have a physical deficit, or when animals are approaching a study endpoint. Professional judgment should be used to ensure that the frequency and character of observations minimize risks to individual animals and do not compromise the research for which the animals are used." the Guide, p. 112

III. Responsibilities

This document applies to all animal users at Rutgers University.

IV. Methods

A. All vertebrate animals must be observed at least once every day including weekends and holidays.

B. In centrally-managed vivaria, CMR staff document the daily observations (basic health and environmental status check). Additional monitoring by laboratory members must be conducted when specified in individual IACUC protocols.

C. In satellite animal facilities, a member of the investigator's laboratory must perform daily observations, complete the log sheet, and comply with the IACUC Policy Satellite Animal Facilities.

V. References

- Guide for the Care and Use of Laboratory Animals, 8th edition, NRC Press, 2011.

RUTGERS UNIVERSITY IACUC Document #G5	Use of Wire-Bottom Cages
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 4/21/2021 (version 3.0)

I. Purpose

The purpose of this document is to provide animals users with guidance when housing animals in wire-bottom cages.

II. Introduction

According to the Guide,[5] "When given the choice, rodents prefer solid floors (with bedding) to grid or wire-mesh flooring. If wire-mesh flooring is used, a solid resting area may be beneficial, as this floor type can induce foot lesions in rodents and rabbits."

While AAALACi does not provide an official Position Statement, AAALACi has made its position clear over the years that use of wire-bottom caging is to be discouraged and must be justified in an approved protocol.

III. Responsibilities

This document applies to all animal users at Rutgers University.

IV. Methods

Use of Wire-Bottom Caging (including metabolic caging)

1. Wire-bottom caging is considered an exception to the Guide and therefore requires strong scientific justification to the IACUC prior to its use.
2. Animals must be housed on wire-bottom caging for the minimum duration necessary to achieve the experimental goals.
3. If possible, a solid resting surface must be provided for animals in each wire-bottom cage.
4. All animals housed in wire-bottom caging must have their feet inspected at least once a week and all observations must be documented in the room logs.
5. Suitable enrichment must be provided for animals housed in wire-bottom caging, unless the IACUC has approved withholding enrichment in the protocol.

[5] Guide for the Care and Use of Animals, 8th ed., 2011, pp. 51, 52.

RUTGERS UNIVERSITY IACUC Document #G6	Enrichment and Social Housing
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 4/21/2021 (version 3.0)

I. Purpose

This document establishes guidelines for providing environmental enrichment to laboratory animals.

II. Introduction

"The primary aim of environmental enrichment is to enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species typical behaviors and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics.[6]"

"An appropriate housing space or enclosure should also account for the animals' social needs. Social animals should be housed in stable pairs or groups of compatible individuals, unless they must be housed alone for experimental reasons or because of social incompatibility.[7]"

III. Responsibilities

At a minimum, all animals must be provided with enrichment and social housing unless approved in the protocol by the IACUC as a departure from the Guide. Both CMR staff and laboratory staff are responsible to ensure species specific enrichment requirements are met.

IV. Methods

A. Routine Enrichment. All animals should be provided standard enrichment based on species. It has been shown that in some instances (e.g., neurological or behavioral studies) enrichment can alter the outcome of an experiment. In such cases enrichment can be withheld, if adequate scientific justification is approved in the IACUC protocol prior to implementation**. Social housing alone is not considered sufficient enrichment.**

B. Examples of acceptable enrichment can include the following:

1. **Burrowing and Nesting.** Rodents housed in solid bottom caging may have materials added to the cage to promote burrowing or nesting behaviors (e.g. Enviropak, Nestlets®, paper rolls, paper towels, nesting sheets, diamond twists, foraging boxes, or additional bedding). In sterile rodent cages any introduced burrowing or nesting materials must be autoclaved or sterilized in accordance with proper husbandry techniques. Larger animals may be provided with straw, hay, or other substrates to promote species-specific behavior.

2. **Food Treats.** Food treats may be provided as part of the enrichment program. Suitable food treats for rodents include a variety of seeds or other treats. Food treats for large animals, depending on

[6] Guide for the Care and Use of Laboratory Animals, 8th ed., 2011. pp. 52-53
[7] Guide, 2011. p. 51.

species, can include such items as fruit, vegetables, and/or species specific treats. In sterile rodent cages, any introduced food treats must be irradiated or sterilized in accordance with proper husbandry techniques.

3. **Foraging.** Food treats may be mixed with bedding or species-specific toys (e.g. Kong toys, puzzle feeders, etc.) to encourage foraging behavior.

4. **Shelters.** PVC tubes, plastic shelves, or plastic/paper shelters (e.g., BioServ® huts and igloos or Shepherd Shacks®) may be placed in cages or tanks to allow animals to seek refuge.

5. **Gnawing.** Nylabones®, Manzanita sticks, wood blocks, plastic chains, cornhusks, cuttle bones, and Critter Cubes® may be used to promote gnawing behaviors.

6. **Manipulanda.** Toys such as mirrors, balls, dumbbells, and hanging chains can be placed in animal enclosures to provide tactile and visual enrichment opportunities.

C. Special Enrichment Considerations Animals that show signs of psychological distress through behavior or appearance must be provided special attention. The Director of CMR or designee will provide specific guidance for increased environmental enrichment for these individuals. The general plan will be to increase the diversity, frequency, and duration of activities normally used to enhance the environment.

D. Social Housing

1. Group housing should be practiced as the default for all social species. Single housing must be justified based on experimental requirements as per the approved IACUC protocol or veterinary related concerns. Social grouping may be suspended upon recommendation and consultation of a CMR veterinarian for health or study-related purposes. In these cases, it should be limited to the minimum period necessary. **If animals are single housed, an extra enrichment item must be offered to all rodents unless in the approved IACUC protocol.**

2. If an investigator routinely group houses all animals and has no justification in the protocol to singly house any animals, the veterinary staff can singly house animals that need to be separated for humane reasons (e.g., animals that have sustained wounds as a result of fighting where their welfare will be compromised if left in the cage with other animals).

3. Each time an animal is singly housed, the cage or tank must be labeled to indicate the reason for single housing. If every housing unit in the room or rack is singly housed, the label may be placed at the room or rack level.

4. **Considerations for Single Housing**

   - Veterinary Exemption
     - Fighting or Incompatibility. Some animals may fight if paired or group housed (e.g., breeder males, some strains of mice).
     - Post-Operative Recovery. Animals can be singly housed in the immediate post-op period as they are often susceptible to injury from cage mates until fully recovered. CMR veterinarians can be consulted for additional guidance.
     - Health Conditions. Animals that experience health conditions that may require single housing to avoid injury from cage mates and being outcompeted for food.

- Other conditions, as approved by a CMR veterinarian

- Study Design - Single housing for experimental reasons must be included in the approved IACUC protocol. Some studies such as nutritional, physiological, behavioral, where individual animal data must be collected may be single housed as per the approved IACUC protocol.

- Breeding
  - Single male or female in litter at the time of weaning in the absence of suitable social housing
  - Breeding stud
  - Pregnant female from a trio or harem breeding scheme

- Research Attrition
  - Remaining animal in an experimental cohort where no suitable cage mate is available

V. References

- Guide for the Care and Use of Laboratory Animals, 8th ed., NRC Press, 2011.

RUTGERS UNIVERSITY IACUC Document #G7	Sanitation and Monitoring of Research Equipment
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 4/21/2021 (version 3.0)

I. Purpose

This document outlines the procedures and responsibilities to assure proper sanitation of restraint, enrichment and other equipment used in animal studies conducted by animal users in laboratories (including satellite facilities) and maintained by research faculty and staff. This does not include equipment maintained by CMR.

II. Introduction

Cleaning and disinfection are necessary to prevent cross-transmission or exposure to microorganisms, excrement, biological fluids, and pheromones from one research subject to another and to remove these substances as well as allergens from work environments shared with humans. When accompanied by scrubbing to remove organics and other soilage, effective disinfection by definition significantly decreases the number of microorganisms from inanimate objects. "Sanitation of cages and equipment by hand with hot water and detergents or disinfectants can also be effective but requires considerable attention to detail. Whether the sanitation process is automated or manual, regular evaluation of sanitation effectiveness is recommended."[8]

In addition, the Animal Welfare Act requires that "used primary enclosures and food and water receptacles must be cleaned and sanitized in accordance with this section before they can be used to house, feed, or water another [animal][9]and that hard surfaces of primary enclosures and food and water receptacles must be sanitized using one of the following methods: live steam under pressure; washing with hot water (at least 180°F (82.2°C)) and soap or detergent, as with a mechanical cage washer; or washing all soiled surfaces with appropriate detergent solutions and disinfectants, or by using a combination detergent/ disinfectant product that accomplishes the same purpose, with a thorough cleaning of the surfaces to remove organic material, so as to remove all organic and mineral buildup, and to provide sanitization followed by a clean water rinse."[10]

III. Responsibilities

Animal users are responsible for disinfection of all equipment and work surfaces in their laboratory that may have come in contact with animals prior to and after use with each animal or group of animals. Animal users are encouraged to keep a log sheet documenting dates of disinfection and sanitation for all equipment. CMR monitors the effectiveness of sanitation of experimental equipment that is currently in use, at least once every three years. Animal users should sanitize any equipment before it goes into storage and once it is removed from storage, prior to use.

[8] Guide to the Care and Use of Laboratory Animals, 8th ed., National Academies Press: Washington. 2011, pp. 71, 73.
[9] Animal Welfare Act (9 CFR Ch.1 §3.11(b) (1)
[10] Animal Welfare Act (9 CFR Ch.1 §3.11(b) (3)

IV. Methods

A. General Sanitation Procedures

All portable and fixed equipment as well as surfaces that come in contact with animals must be cleaned and disinfected prior to and after each use by the personnel using the equipment and/or procedural areas (e.g., imaging equipment, behavioral testing apparatus, surgical equipment, animal restraint device, countertop and work surface). Disinfection is most effective when all visible dirt and debris are removed prior to application of the disinfectant. Any bedding used in chambers must be removed prior to sanitation and replaced with fresh, clean substrate following testing. Bedding which comes in direct contact with animals should be changed between animals. Sanitation should be performed between animal cohorts.

B. Options for Sanitation

1. Sanitizing in a mechanical washer (preferred)
2. Handwashing, primarily for housing enclosures not conducive to mechanical washing
3. Manual wiping with disinfectant is most appropriate for fixed surfaces, stationary equipment and delicate, heat- or moisture-sensitive apparatus.

C. Recommended Disinfectants – Follow manufacturer recommended preparation guidelines and contact times for each agent. As an additional reference, review REHS biosafety guide on disinfection (Table 5).

Agent	Examples	Comments
Alcohols	70-85% ethyl alcohol; 70-85% isopropyl alcohol; Hand gel sanitizers	Remove gross contamination before using. Inexpensive. May damage rubber and plastic items. Alcohols are only acceptable if no other agents can be used on a specific piece of equipment.
Quaternary Ammonium	Roccal®, Quatricide®, CONFLIKT	Safe, generally effective general environmental disinfectant. Rapidly inactivated by organic matter and other environmental materials. May support growth of Gram-negative bacteria. Should not be used near breeding animals.
Chlorine	Sodium hypochlorite (Clorox ®), Chlorine dioxide (Clidox®, Alcide®, MB-10®)	Highly effective. Corrosive and oxidizing. Presence of organic matter reduces activity. Chlorine solutions must be fresh because they degrade over time.
Phenolics	Lysol®, TBQ® VespheneTM	Broad spectrum activity. Less affected by organic material than other disinfectants. Some may be corrosive, harsh, toxic with a pungent odor. Skin irritant.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent, but bacterial spectrum is narrow. Effective against many viruses.
Hydrogen peroxide, peroxygenated compounds	Virkon-S®	Broad spectrum of activity. Environmentally safe. Powder is corrosive. Wipes are recommended.
Peracetic acid	Minncare®	Broad spectrum activity. Corrosive in pure form. Biodegradable. Often requires special equipment and/or expertise. Most useful for medical and surgical devices. Strong, pungent odor. Respiratory irritant, and can only be used in DUCTED cabinets.

D. Cleaning Recommendations

1. Restrainers and Enrichment Devices
   a. Wash and rinse in a mechanical cage washer, or
   b. Hand wash
      i. Wash used restrainer or device to remove all debris.
      ii. Soak or spray equipment with a suitable sanitizer (see table above).
      iii. Soak or let sit (for sprayed items) for at least the minimum contact time for the product used.
      iv. Rinse with clean water, allow to dry.

2. Test Chambers
   a. Remove all loose bedding, feces and other debris from chamber.
   b. Wipe down or spray all surfaces with one of the recommended cleaning agents and allow to sit for the minimum contact time.
   c. Wipe down surfaces with clean water and allow to dry.

3. Stereotaxic and other Surgical, Technical, or Experimental Equipment
   a. Remove all debris from device.
   b. Disinfect all points of contact with an animal.
   c. Surgical instruments must be sterilized before use and maintained using aseptic technique (see IACUC Policies Non-Rodent Surgery / USDA Species and Non-Rodent Surgery / USDA Species for further information).

E. Sanitation Contraindications

1. It is not recommended that chlorine dioxide-based disinfectants/sterilants be used on stainless steel equipment and surfaces unless it is cleaned off thoroughly with water.
2. If residual odors from the cleaning chemicals might affect study animals, or if chemicals may damage equipment, an exemption from equipment sanitization must be justified in the approved IACUC protocol.

Quaternary ammonium compounds should be used with caution around breeding animals, as studies have demonstrated decreased fertility in rodents exposed to these chemicals.

RUTGERS UNIVERSITY IACUC Document #H1	Photography and Videography of Laboratory Animals
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 5/19/2021 (version 3.0)

I. Purpose

This document provides guidance and regulation of photography and videography of live or dead laboratory animals. There are three distinct categories of photography/videography:

1. As required to achieve the scientific goals of the research program
2. Done for purposes of documentation and review not related to research goals (e.g., Post-Approval Monitoring and/or regulatory compliance)
3. Videos for training and/or dissemination of technique

Photography and videography for scientific research are generally permitted, but must be described in the animal protocol. The protocol should describe 1) details of the collection of the video, 2) its role in achieving the scientific goals, and 3) the dissemination of video data.

Photography and videography for documentation, training, or any purpose not related to achieving the scientific goals of research require prior approval from either CMR or the IACUC as described below.

This document does not apply to the use of security cameras placed in animal facilities to monitor animal and human safety.

II. Introduction

There is a legitimate need for photography of laboratory animals at Rutgers University in the conduct of research, to document clinical disease, staff non-compliance, research practices and techniques (e.g., videos for training and/or dissemination of methods), and potential documentation by outside regulatory agencies.

III. Responsibilities

This document applies to all animal users that use photographic/videographic animal data in their research, and all parties that may photograph or video animals for non-scientific purposes including documentation.

IV. Definitions

Animal Facility - Any CMR-operated or satellite animal holding area. This does not include the Rutgers School of Environmental and Biological Sciences (SEBS) agricultural facilities on the Cook Campus farm.

Investigator Research Areas - Any room or area outside of a centralized or satellite facility where there is Rutgers IACUC oversight of animals used in research, testing, or training.

Device - Includes cameras (digital or film), video recorders, camera phones, webcams, tablet computers, laptops, tape/audio recorder, and any similar devices with recording capabilities (video and/or audio).

Recording - Any photograph, file, image, tape, or video created by a Device.

Visitor - Any individual without assigned key or keycard access to a specific Animal Facility or individual visiting an investigator research area who is not listed in an IACUC protocol specific to that location.

- See IACUC Policy Visitors to Rutgers Animal Facilities

V. Methods

A. Scientific photography and videography

Animal research often requires the collection of photographic or videographic data. Examples include kinematic and biomechanical studies, behavioral studies, ecological research, social research, and studies of animal movement in the environment.

The collection of photographic and videographic data must be described in the animal protocol. This description should include the scientific rationale, a description of the images (what will be seen in the images, the status of the animals, relation to surgical or other potentially painful/stressful manipulations, etc.), the measurements that will be made in the photographic/videographic data, and the potential dissemination of the photographic/videographic evidence.

Special care must be taken to disseminate evidence that captures the scientific conclusions while minimizing its potential misuse. Investigators are strongly recommended to submit photographic evidence or videos of animals for review by either the IACUC or CMR, as appropriate, before publication.

B. Requirements for photography and videography

1. Recordings must show appropriate and accurate context.
2. Appropriate personal protective equipment must be worn by all persons in the recording.
3. Animals should be shown in clean surroundings with unnecessary items removed. Access to water and food should be visible in the recording whenever possible.
4. References to personal information must not be visible. Attention should be paid to surrounding areas and items such as cage cards.
5. Recordings of personnel require approval by each individual recorded. Official Rutgers release forms should be used if intended for public viewing.
6. Appropriate handling and restraint methods for the species must be used.
7. Only the smallest portion of the animal, surface, or room may be shown.
8. Encrypted file transfer is strongly recommended for the transmission of these images/videos.
9. Whenever possible, recordings should be taken on or transferred to password-protected devices and deleted from all non-password protected devices.
10. Recordings taken in irradiation facilities or select agent use areas need additional approval by Rutgers Environmental Health and Safety (REHS).
11. **Recordings must not be posted on any social media or public websites.**

C. Photography/videography for CMR and/or IACUC documentation and education

1. Recordings may be made for educational seminars, clinical diagnoses, documentation of non-compliance, or post-approval monitoring (PAM).
2. Recordings used for training purposes must not have any reference to the PIs or the facility.
3. An animal care manager, supervisor, or member of the veterinary staff must be present for all recordings.

D. Photography/videography by Visitors

1. Rutgers University will consider reasonable requests to visit its animal facilities, research, and teaching laboratories. However, in order to protect the confidentiality of faculty research, to provide a minimally disruptive atmosphere for the animals, and to guard against the misinterpretation of appropriate and humane policies and procedures, photography and/or audio/video recording is not allowed except for official purposes that are approved explicitly by the CMR Director or designee.

    a. Visitors are not permitted to take recordings in the animal facility except: (1) government inspector and photo documentation is necessary for official duties or (2) visitor is serving as a photography vendor for the faculty - all such vendor photo documentation is subject to the points listed above and must be approved by the CMR Director or designee and the appropriate REHS Responsible Official, as applicable. Only IACUC approved procedures can be recorded.
    b. A CMR or IACUC Office staff member must advise visitors concerning the prohibition of photography in conjunction with any request for a visit and at the time of entrance into the animal facility.
    c. An animal care manager, supervisor, or member of the veterinary staff must be present for all recordings being taken.

2. When performed by government inspectors (e.g. USDA Veterinary Medical Officer) or other regulatory officials conducting a site inspection who elect to take recordings, the visitor must agree to the following conditions:

    a. Recordings can be taken only as an official part of the inspection.
    b. Recordings used in a report must be provided to CMR Director or designee and the REHS Responsible Official, as applicable, for review and approval, as well as all other components of site inspection reports.
    c. Recordings will not be distributed or used in any way other than as supporting evidence for an official site inspection report.
    d. When recordings are used to document deficiencies, equivalent recordings will be taken by CMR staff to document corrections and both may be used together in any report of non-compliance. CMR staff may request inspectors to take additional photographs for fairness.
    e. USDA Veterinary Medical Officers must allow the Institutional Official or designee to review or redact the records for proprietary business information, research facility records, protocols, or IACUC minutes. The USDA inspector must allow the facility 24 to 48 hours for this purpose.[11]
    f. The CMR Director or designee must review all recordings before release, and may require that these recordings be destroyed.

E. Videos for training and/or dissemination of techniques (e.g., JoVE)

1. Videos may be made by CMR staff or by investigators for the purpose of training and/or dissemination of techniques. These videos have an important scientific function but are not necessary for the scientific goals of the research (as described above).
2. A script for the video should be provided to the CMR Director or designee in advance of filming to allow a congruency review with the protocol. The CMR Director or designee will ensure appropriate additional review as required (e.g., veterinary review and media relations review and REHS Responsible Official, as appropriate).

[11] Animal Welfare Inspection Guide v. 12/7/2021, section 2.5.1, 7.5.6

3. Videography staff must adhere to IACUC Policy Visitors to Rutgers Animal Facilities and be escorted by approved research staff at all times.
4. Filming should only capture laboratory areas and animals as needed for training/education.
5. Where possible and if the lab location is approved in the protocol, arrangements for filming should be made to occur outside of a designated animal facility and ultimately performed as a terminal procedure.
6. Researcher must provide the CMR/IACUC Director or designee with the finalized video for review in advance of publication. The CMR/IACUC Director or designee will ensure appropriate additional review as required (e.g., veterinary review and media relations and REHS Responsible Official, as appropriate).

RUTGERS UNIVERSITY IACUC Document #H2	Animal Welfare Concern Reporting (Whistleblower)
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 4/21/2021 (version 3.0)

I. Purpose

Rutgers University is committed to the humane treatment of all animals used in research, testing, teaching and production. This guideline provides direction on animal welfare concern reporting.

II. Introduction

"The institution must develop methods for reporting and investigating animal welfare concerns, and employees should be aware of the importance of and mechanisms for reporting animal welfare concerns."

- the Guide, 2011

"No facility employee, committee member, or laboratory personnel shall be discriminated against or subject to any reprisal for reporting violations."

- AWA (9 CFR Ch.1), Part 2 - Subpart C, 2.32.2

III. Responsibilities

This document applies to all visitors, students, staff, faculty, animal users, and employees of Rutgers University.

IV. Methods

Rutgers University is committed to the humane care and use of all animals used for research, teaching, testing, and production. If an individual or group of individuals see or know of activities which they believe constitute inappropriate animal care or use, they are obligated to report such activities. Inappropriate care and use may include but is not limited to inhumane treatment, abuse, neglect, unapproved procedures, etc. The IACUC is required by the federal Animal Welfare Act and the Office of Laboratory Animal Welfare (OLAW) to deal with such reports in a confidential manner and to investigate them fully. The regulations prohibit discrimination or reprisal against any person or persons for reporting violations.

Signage describing the procedures for reporting animal welfare concerns must be posted in all animal use areas.

All reports made directly to the CMR staff and/or the IACUC will remain confidential. See below for additional routes of reporting in which the reporter can remain anonymous.

All concerns are thoroughly investigated regardless of how they are reported, including anonymous reports.

Concerns regarding animal care and use may be reported to any of the following:

Attending Veterinarian (AV)
IACUC Chair
CMR/Animal Care Management
IACUC Office staff
Institutional Official (IO)
Any IACUC member

Any questions or concerns can also be sent to:
animalconcerns@rutgers.edu (sender email address will be visible)
or submitted by telephone to:
Rutgers Compliance Hotline: 1-833-783-8442 (can remain anonymous)
or online at:
https://uec.rutgers.edu/compliance-hotline/ (can remain anonymous)

V. References

- The Guide for the Care and Use of Laboratory Animals, current edition
- The Animal Welfare Act
- Department of Health and Human Services, Office of Research Integrity Guidelines for Institutions and Whistleblowers: Responding to Possible Retaliation Against Whistleblowers in Extramural Research (November 20, 1995).

  https://ori.hhs.gov/guidelines-whistleblowers

RUTGERS UNIVERSITY IACUC Document #H3	Visitors to Rutgers Animal Facilities
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 5/19/2021 (version 3.0)

I. Purpose

This document provides guidelines for all individuals entering Rutgers University animal facilities.

II. Introduction

Visitors may enter animal facilities for a number of reasons including but not limited to collaborative scientific effort, an educational effort, to assist in fund raising, vendors, maintenance staff or to promote awareness of the need to use animals for scientific discovery. This document is intended to protect the research animals, research endeavors, and the visitors themselves, while also preventing disruption to research activities.

III. Responsibilities

Any Rutgers personnel responsible for escorting or providing access to a visitor must assure that every visitor adheres to this policy.

IV. Definitions

Animal Facility - Any CMR-managed or satellite area that houses vertebrate experimental animals. Animal facilities may include animal housing areas, procedure areas, storage rooms, office space, and hallways. This excludes the Cook Campus farm.

Laboratories are spaces where experimental animals are temporarily kept and where procedures may be performed consistent with IACUC approved protocols. Access by visitors to laboratory areas, including laboratory areas where animals are temporarily kept, may be granted by and is the responsibility of the PI.

Minors are defined as all persons under 18 years of age except for minors enrolled at Rutgers who are participating in laboratory activities as part of their normal coursework and minors who are employees of Rutgers. Students and employees who are under 18 years of age engaged in animal research must comply with the same regulations that govern all other students and employees.

Visitors include anyone not authorized under an approved Rutgers University IACUC protocol to enter an animal facility, observe animal research, or have direct contact with laboratory animals as part of their specific academic position or job responsibilities at Rutgers University or by law or regulation.

Visitors do not include:

- Employees, fellows, or students of the University authorized to participate in one or more IACUC approved protocols.
- A consultant or other non-Rutgers University faculty, staff, or student registered as an authorized agent or guest and approved for work on a specific IACUC approved protocol.
- An outside vendor or a University employee not otherwise approved on a specific IACUC approved protocol but authorized by CMR for the purpose of training, facility maintenance, or inspection.

Guide is either Animal Care (including CMR) staff, the PI, or designated IACUC protocol personnel.

V. Methods

A. Visitors to Animal Facilities

Visitors may be permitted in animal facilities or research laboratories where animals are used if approved in advance by CMR veterinarians and/or supervisory staff in compliance with this policy, and the visitor is accompanied at all times by the guide. Visitors to BSL3 facilities require REHS approval. REHS approval may be required in other facilities.

Faculty should also notify a member of CMR supervisory staff at least 5 business days in advance that they will sponsor a first-time visitor to an animal use area. The guide should provide CMR with the visitors' names, the reason for the visit, the planned visit time, and facility or laboratory location by e-mail or in writing. For visitors who wish to enter rooms where animals are housed, Occupational Health requirements must be met, where applicable. Occupational Health clearance for the visitor will be accepted from their home institution and documentation must be provided upon request.

It is the responsibility of the guide to inform the visitor that animal facilities may pose health risks to individuals who have allergies to animals or animal dander, or those who are immunocompromised. Such persons should sign the visitor log book in each facility, if applicable, and be advised to avoid entering animal facilities while animals are housed or to take all appropriate precautions to avoid or limit exposure. It is also the responsibility of the guide to discuss with the visitor the risks that he or she may pose to research animals. It is also the responsibility of the guide to assure compliance with IACUC Policy Photography of Laboratory Animals or any other applicable guidance or clearance requirements (e.g. international visitors must obtain clearance from Export Control, Office for Research). Persons who have active communicable diseases of public health concern (e.g. tuberculosis, influenza, COVID-19, or other respiratory diseases) are not permitted to visit animal facilities.

Persons who have been in non-Rutgers animal facilities within the last 48 hours may not enter a Rutgers animal facility, unless cleared by the Director of CMR or designee beforehand.

Visitors may enter animal facilities, tour hallways, and/or view animals through doorways without entering animal housing or procedure areas without prior occupational health screening.

Visitors who wish to enter animal housing or procedure areas while animals are present must participate in occupational health screening in advance of the visit as is required for Rutgers employees. All visitors must follow facility rules regarding gowning and donning personal protective equipment.

Persons who intend to handle animals on a research protocol must complete the required orientation training and be added to that protocol. These individuals are not considered visitors.

Public tours through animal facilities are generally not permitted but if allowed, tour participants' access to animal facilities is limited to business areas and/or viewing of animals through door windows. If a public tour is planned, this must be arranged with written documentation and consent of the CMR Director and/or the Senior Vice President for Office for Research or their designee. Such a tour generally may only be initiated in consultation with the IACUC.

B. Visitors to Laboratories

Visitors to laboratory areas where animal research procedures are being performed will fall into one of two categories: those who will observe the procedure, and those who will participate in performing the procedure. These visitors are governed by all the points above, plus the following:

1. Visitors Observing Animal Procedures:
   The PI must provide documentation that occupational health requirements for this visitor have been met either through Rutgers' Occupational Health program, Rutgers' Student Health Center, or that of another institution if the visitor is from outside of Rutgers.

2. Visitors Performing Animal Procedures:
   a. Persons who intend to handle animals on a research protocol must complete the required orientation training and be added to that protocol. These individuals are not considered visitors.
   b. Vendors who will perform animal procedures for demonstration purposes are generally not required to complete orientation training or be added to a protocol. CMR will evaluate on a case by case basis.

C. Minor Visitors to Animal Facilities or Laboratories

1. The presence of minors in a biomedical research laboratory must have a defined research or educational purpose. Minors are not allowed to have physical contact with live laboratory animals unless an exception has been approved by Risk Management and REHS, but they can observe animal research with the following limitations:
   a. No one under the age of 15 is allowed to enter animal facilities
   b. Some facilities may exclude all minors with no exceptions

2. Minors who wish to observe animal research must meet the following requirements in full:
   a. The Institutional Policy on Minors in Laboratories has been read and signed by the faculty member/researcher with responsibility for activities in the laboratory.
   b. The minor's parent or legal guardian has read and signed a Consent for a Minor in Laboratories.
   c. The minor has completed safety training approved by the Rutgers Environmental Health and Safety Office.
   d. The minor is directly supervised by the faculty member/researcher or designated supervisor at all times while in the laboratory.
   e. A Risk Management registration form for minors present in laboratories that describes the educational objective of the experience has been completed.
   f. Any special requests or deviations to this document must be considered and approved by the IACUC.

D. Summary of Requirements based on Type of Visitor

		Requirements:		Approval from:	
Description of Visitor(s)	**Handle Animals?**	**Training**	**Occ Health**	**CMR**	**IACUC and/or IO**
1. Vendor reps performing equipment demos using live animals	Y	N	N*	Y	N
2. Vendors needing to access animal facilities to install or repair equipment in animal facilities (e.g. HVAC contractors)	N	N	N*	Y	N
3. Vendors bringing in potential customers to see equipment purchased and installed at Rutgers.					
a. Under a formal agreement at the time equipment was purchased	N	N	N*	Y	N
b. Under an informal agreement	N	N	N*	Y	N
4. a. Non-Rutgers students, interns, externs, volunteers, visiting scientists working with faculty researchers or CMR (e.g. veterinary students, college students, vet tech students, high school students) who will have contact with animals	Y	Y	Y	N	Y
b. Non-Rutgers students, interns, externs, volunteers, visiting scientists who will not have contact with animals	N	N	N	Y	N
5. Animal facility tours for Rutgers faculty, staff, and students not formally in the animal care program and who do not have orientation training or occupational health approval. (e.g. Biomedical Engineering students in a careers course, Rutgers University President, faculty candidates)	N	N	N	Y	N
6. Animal facility tours for non-Rutgers personnel					
a. Potential contract customers who are considering doing animal work in Rutgers facilities	N	N	N	Y	N
b. Media/legislator tours	N	N	N	Y	Y
7. Attendees at a workshop taught at Rutgers (e.g. Keck Center workshops)	Y	Y**	Y	N	Y
8. Non-Rutgers consulting clinical care providers or veterinarians	Y	N	N*	Y	N
9. Continuing Medical Education (CME) students	Y	Y**	Y	N	Y

*Occupational Health risks assessed by the vendor's or care provider's employer.
**Animal Care and Use orientation training is included as part of the course or workshop curriculum.

VI. References

- Rutgers University Policy – Protection of Minors #30.1.9: https://policies.rutgers.edu/view-policies/administration-and-public-safety-%E2%80%93-section-30

RUTGERS UNIVERSITY IACUC Document #H4	Adoption of Laboratory Animals
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 5/19/2021 (version 3.0)

I. Purpose

This document describes procedures for the adoption of laboratory animals by a private owner.

II. Introduction

The Guide for the Care and Use of Laboratory Animals, and the Guide for the Care and Use of Agricultural Animals in Research and Teaching discuss the termination of research animals at the conclusion of the research project. However, these documents, the PHS Policy and the Animal Welfare Act provide no guidance on the private adoption of research animals.

OLAW is supportive of the concept of adoption but NIH grant funds may not be used to support the cost of the program. The PHS will not assume legal or financial responsibility for any adoption program or any results of adoption. The institution should ensure that its policy meets pertinent state and local regulations for transfer of animal ownership and is encouraged to coordinate with local animal shelters.[12]

III. Responsibilities

The IACUC is responsible for approval of animal adoptions. Adoption is done in consultation with the PI and at the discretion of the Attending Veterinarian or designee. Once the animal leaves the Rutgers campus, all animal welfare including subsequent health checks and veterinary care is the sole responsibility of the new owner. Rutgers University assumes no obligation or responsibility for animals once the adoption process is complete.

IV. Methods

A. The PI must state that the animal is no longer needed for research, has no knowledge that would make the animal inappropriate for adoption, and recommends the animal be offered for adoption.
B. The PI, in conjunction with a CMR veterinarian, reviews the minimally required criteria for eligibility for adoption:
    1. Relevant health and procedure history must be disclosed to the potential adopter.
    2. The animal must not have been administered any substances other than FDA-approved human or veterinary drugs, food supplements or pharmaceutically compounded veterinary drugs.
    3. Animals that have been exposed to hazardous or infectious agents are ineligible for adoption.
    4. Transgenic and immune-suppressed animals are ineligible for adoption.
    5. Animals must not be used for animal or human consumption (food).
    6. The animal must in good health and acceptable behavior, as determined by a CMR veterinarian.
C. Animals can only be adopted if they are not needed for further use by the University.
D. A CMR Veterinarian conducts a health and welfare exam and administers vaccinations, when indicated. Appropriate clinical records may be provided to the potential adopter.

[12] OLAW website, FAQ #11: https://olaw.nih.gov/guidance/faqs#useandmgmt_11 (2018-12-5)

E. A CMR Veterinarian or designee discusses the reasons for the adoption and evaluates the adopter's ability to provide for the animal's welfare.
F. The AV or designee reviews the documentation and may consult the IACUC Office and/or IACUC Chair prior to approval.
G. A completed Animal Adoption Form must be submitted to the IACUC Office. A CMR veterinarian and/or IACUC Chair may restrict the direct adoption of any individual animal to the public, requiring that they only be placed via animal placement groups that keep the source of the animal confidential.

RUTGERS UNIVERSITY IACUC Document #H5	Designated Member Review
	Date Issued: 10/7/2015 (version 1.0) **Date Revised:** 5/19/2021 (version 4.0)

I. Purpose

This document describes the designated member review (DMR) process for the review of IACUC submission at Rutgers University.

II. Introduction

The Office of Laboratory Animal Welfare (OLAW) Frequently Asked Questions (FAQ) D.3 states: "Designated member review may be utilized only after all members have been provided the opportunity to call for full-committee review. If any member requests full committee review, then that method must be used. If not, the IACUC Chairperson may appoint one or more appropriately qualified IACUC members to serve as the designated reviewer(s). Designated review may result in approval, a requirement for modifications (to secure approval), or referral to the full committee for review. Designated review may not result in withholding of approval."

III. Responsibilities

The IACUC members, IACUC Office, and PIs are responsible for complying with policies and procedures outlined in this document.

IV. Methods

A. Designated Member Review in Lieu of Full Committee Review (DMR in lieu of FCR)

1. Categories of protocols routed to DMR in lieu of FCR

a. **USDA**:
   i. New and Triennial USDA protocols at stress levels "C"
   ii. All Annual Reviews for USDA protocols, regardless of stress level
   iii. Amendments to USDA protocols that do not raise the stress level to "E"

b. **Non-USDA**:
   i. New and Triennial non-USDA, non-rodent protocols at stress levels "C" and "D"
   ii. New and Triennial non-USDA, rodent protocols, regardless of stress levels
   iii. Amendments to non-USDA, non-rodent protocols that do not raise the stress level to "E"
   iv. Amendments to non-USDA, rodent protocols, regardless of stress level
   v. All Annual Reviews for non-USDA protocols, regardless of stress level

c. No other categories of protocols may undergo DMR in lieu of FCR

d. PI responds to questions at the time of submission to determine eligibility for DMR in lieu of FCR. PI determined eligibility is subject to IACUC review.

2. Procedure for DMR in lieu of FCR

a. IACUC members are notified of all protocols routed to DMR in lieu of FCR and have access to all protocols undergoing DMR.

b. All IACUC members are given the opportunity to comment on the protocol and/or call for FCR. The objection period is one day, although all IACUC members have access to review the protocol and call for FCR throughout the review process. If an IACUC member calls for FCR of a protocol during the objection period, that protocol is placed on the next IACUC meeting agenda.
c. If FCR is not requested by any member of the IACUC, at least one member of the IACUC, selected from a list provided by the Chair and qualified to conduct the review, reviews the protocol and has the authority to approve, require modifications (in order to secure approval) or request FCR for the protocol.
d. If the PI has not responded to questions from the IACUC within 60 days, the submission may be administratively withdrawn.
e. The approval date is the date that the designated member(s) approve the study. No animal work can begin until the Notice of Approval is received by the PI. Animal work conducted before this date is considered a serious noncompliance of PHS policy IV.F.3 (PHS Policy IV.F.3), and reportable to OLAW.

B. Designated Member Review Subsequent to Full Committee Review (DMR following FCR)

1. Categories of protocols that may undergo DMR subsequent to FCR

a. Any type or stress level protocol may undergo DMR following FCR

2. Procedure for DMR subsequent to FCR

a. When a protocol is reviewed at a full committee meeting and it is determined that further modification of the protocol is required to secure approval, the quorum of the members present at that convened meeting can decide by unanimous vote, to complete the approval process through DMR. However, any member of the IACUC, including those not present at the full committee meeting can at any time review the protocol and request FCR.
b. DMR reviewers assigned following FCR are the same as those that were assigned to review the protocol at the full committee meeting, unless otherwise specified by the Chair (or the Chair's designee).
c. If the PI has not responded to questions from the IACUC within 60 days, the submission may be administratively withdrawn.
d. The approval date is the date that the designated member(s) approve the study. No animal work can begin until the Notice of Approval is received. Animal work conducted before this date is considered a serious noncompliance of PHS policy IV.F.3 (PHS Policy IV.F.3) , and reportable to OLAW.

V. References

- http://grants.nih.gov/grants/olaw/faqs.htm#615
- http://grants.nih.gov/grants/guide/notice-files/NOT-OD-09-035.html
- http://grants.nih.gov/grants/olaw/references/laba02v31n9.htm

RUTGERS UNIVERSITY IACUC Document #H6	IACUC Review of Protocol Amendments
	Date Issued: 7/10/2018 (version 1.0) **Date Revised:** 5/19/2021 (version 4.0)

I. Purpose

The purpose of this document is to define how changes to a protocol are reviewed and processed.

II. Introduction

Most significant changes to a protocol undergo IACUC review. However, guidance from OLAW provides the IACUC with some discretion to handle specific protocol changes in accordance with IACUC approved policies through consultation with a veterinarian authorized by the IACUC or through changes reviewed by the IACUC office.

III. Responsibilities

Submitted amendments will be routed to one of the following processes:

a. IACUC review,
b. Consideration via VVC by a IACUC member veterinarian in lieu of either FCR or DMR, or
c. Administrative approval as per the requirements of this policy.

Note: For b (VVC), the veterinarian is not conducting a designated member review but is serving as a subject matter expert to verify that compliance with the IACUC-reviewed and approved policy is appropriate for the animals in this circumstance. The veterinarian must refer any request that does not meet the parameters of the IACUC policies to the IACUC and may refer the request to the IACUC for review for any reason.

All IACUC member veterinarians are responsible for reviewing their assigned VVC amendments and are selected for review of individual amendments in rotation by the IACUC office.

IV. Definitions

DMR – Designated Member Review

FCR – Full Committee Review

VVC – Veterinary Verification and Consultation

V. Methods

a. Significant changes requiring IACUC review
   i. The following changes must be approved by the IACUC, either through FCR or DMR: Change from non-survival to survival surgery.
   ii. Addition of a new procedure, or a change in an existing procedure resulting in greater pain, distress, or degree of invasiveness.
   iii. Change in housing and or use of animals in a location (such as an investigator laboratory) that is not part of the animal program normally overseen by the IACUC.
   iv. Change in species.

v. Change in study objectives.
vi. Change in Principal Investigator.
vii. Changes that impact personnel safety.
viii. Addition of a new compound **of a different class** than those already approved in the protocol.
ix. Addition of a non-pharmaceutical drug including those that have been approved in a pharmaceutical formulation.
x. More than 50% increase in the total number of animals required for stress level D or E protocols.
xi. Change in food or water administration.
xii. Change in post-procedural monitoring.
xiii. Change in restraining methods.

b. Veterinary Verification and Consultation - **The VVC process cannot be used for any change that increases the pain or distress to an animal or impacts personnel safety considerations.**

The following changes may be administratively handled according to IACUC policies and guidelines with verification by, and in consultation with, an IACUC member veterinarian:

i. Changes in drugs used for anesthesia, analgesia, or sedation that follow referenced dosages included in the IACUC Policy Anesthesia and Analgesia in Laboratory Animals(pharmaceutical grade drugs only).
ii. Change in euthanasia to any method approved in the AVMA Guidelines for the Euthanasia of Animals (exception: addition of perfusion where previously there was no requirement for anesthesia).
iii. Increase in animal numbers 10% - 50% due to unforeseen circumstances (justification required; e.g. change in statistical analysis) or increased production of offspring in a breeding colony.
iv. Increase in animal numbers <10% for stress level D and up to 100% for stress level B and C USDA protocols
v. Addition of a non-hazardous new experimental compound in the same class of compounds as those already approved in the protocol.
vi. Change in dosing schedule, change in procedure time points, and/or extension of the experiment time if the change does not result in greater pain, distress, or degree of invasiveness.
vii. Change in an existing procedure resulting in less pain, distress, or degree of invasiveness. All procedure changes must conform with approved IACUC Policies. Example: Change in a blood collection method to a less painful or invasive method that is in compliance with the IACUC Policy Fluid Administration and Collection in Rodents.
viii. Addition of a new genotype, strain, or breed of animal provided it does not present with a known adverse phenotype or change the pain category of the protocol, change the objectives of the study, or where new procedures will be added.

VVC NOTE: If a PI requests an emergency change in a drug for anesthesia, analgesia or sedation, or in a dosing schedule:

1. The veterinarian verifies the emergency change fits the VVC policy guidelines and conforms to all Rutgers IACUC policies, after consultation with the PI.
2. The PI must then submit an amendment which is reviewed by the consulted veterinarian and verified through the eIACUC system to be able to continue to use the substitution in any further study.

c. Administrative changes reviewed by IACUC office

Administrative changes that do not require IACUC review or veterinary verification and consultation include:

i. Change of the protocol title
ii. Addition or removal of personnel, other than the PI
iii. Correction of typographical errors and grammar
iv. Increase in animal numbers <10% for stress level D and up to 100% for stress level B and C non-USDA protocols

VI. References

- OLAW Notice #NOT-OD-14-126 https://olaw.nih.gov/guidance/significant-changes.htm
- Rutgers University IACUC Policy Handbook

RUTGERS UNIVERSITY IACUC Document #H7	Subaward Policy
	Date Issued: 8/18/2021 (version 1.0) **Date Revised:**

I. Purpose

The purpose of this policy is to provide guidance and expectations to Rutgers investigators who contract or engage a second party to perform some or all of animal work included in the original award.

II. Responsibilities

Rutgers is considered the Prime awardee under this arrangement and the Rutgers investigator is responsible for complying with this policy. All parties are responsible for complying with the terms of the MOU.

III. Definitions

Grant congruency – Agreement between the animal activities described in a grant and the animal activities reviewed and approved by the IACUC.

MOU – Memorandum of Understanding; for the purpose of this policy the MOU is an official agreement between two institutions which outlines expectations for animal research occurring at the Sub-awardee's location. It also formalizes communication, assigns roles, clarifies animal ownership, and specifies expectations regarding animal-related activity at the Sub-awardee's location (e.g. reporting non-compliance, unexpected outcomes, or change in accreditation status)

Prime awardee (aka Prime) – The Principal Investigator and/or Institution named on a research award; for the purposes of this policy Rutgers University is considered the Prime Awardee.

PI – Principal Investigator

Sub-awardee (aka Sub) – The entity contracted or engaged by the Prime awardee to perform animal research. Commercial vendors of animals or animal products (i.e., antibodies) are not considered Subs.

IV. Policy

A. Sub – The Sub must meet all relevant laws and regulations required to legally perform animal research in the country where the work will be performed. In the United States of America this includes adherence to the Public Health Service (PHS) Policy and the Animal Welfare Act (when applicable). For all federally funded work, the Sub must have a current PHS assurance and accreditation. For all other sources of funding (e.g. foundations, gifts, etc.), the requirements will be evaluated on a case-by-case basis by the Rutgers IACUC Office.

B. Disclosure – The PI must inform the Rutgers IACUC Office in writing whenever animal research work is sub-contracted to another institution prior to the initiation of the work with enough time to allow for sufficient review. No work can commence until approval is given by the Rutgers IACUC Office, regardless of whether the work has been approved by the IACUC of record of the other institution.

C. Initiation of MOU between Prime and Sub – If a Prime / Sub arrangement is being considered, an MOU must be created for each protocol prior to the initiation of the work. A template MOU which should be used is attached to this policy. No work may be conducted at the Sub until Rutgers has executed the MOU

with the Sub. Any work conducted prior to execution of the MOU shall be considered work done without IACUC approval.

D. Grant congruency – NIH Grants Policy Statement (NIHGPS) "It is an institutional responsibility to ensure that the research described in the application is congruent with any corresponding protocols approved by the IACUC." Rutgers' expectation is for the Sub to perform grant-protocol congruency review. However, at the discretion of the IACUC Office, Rutgers may conduct grant-protocol congruency review on a Sub's protocol. The Rutgers PI is required to provide Rutgers with the Sub's IACUC Protocol Notice of Approval.

E. Signing of MOU - The MOU will be signed by the Rutgers Institutional Official or their designee or IACUC Director as a representative of the Prime awardee.

F. Responsible party – Rutgers University and the PI identified on the original award are ultimately responsible for all animal work, including work performed at the Sub's location.

G. Rutgers University as Sub-Awardee
   1. If Rutgers University is named as a Sub, the Prime will be expected to originate the MOU.
   2. If Rutgers University is named as a Sub- and subcontracts work to a third party, Rutgers will be considered the Prime for that portion of the award.

RUTGERS UNIVERSITY IACUC Document #H8	Compliance Concerns Involving the Care and Use of Animals
	Date Issued: 8/18/2021 (version 1.0) **Date Revised:**

I. Purpose

The purpose of this document is to describe the methods to investigate, report, and resolve potential compliance concerns with respect to animal care and use.

II. Introduction

Compliance concerns including those relating to any use of animals in research, teaching, testing, or production at Rutgers University may be brought to the IACUC by the public, faculty, students, or staff. Concerns may include alleged instances of animal mistreatment, violations of approved protocols, animals used without approved protocols, changes instituted to an approved protocol without prior submission and approval of a required amendment, violations of any animal welfare related regulation or policy (e.g. PHS Policy, AAALAC International, Animal Welfare Act, or Rutgers Animal Welfare Assurance), and/or complaints regarding the care received by animals housed in University laboratory animal, wild animal, or agricultural facilities.

III. Responsibilities

It is the responsibility of every animal user to follow all applicable rules, regulations, and standards pertaining to the use of animals for research, teaching, testing, or production at Rutgers University. The IACUC will investigate and determine the appropriate response to cases of confirmed non-compliance.

IV. Definitions

Executive Committee - The IACUC Executive Committee is a subcommittee of the IACUC and is typically comprised of the IACUC Chair, Vice-Chair, AV, CMR Veterinarians, IACUC Office Director, IACUC Office Assistant Director, Compliance Administrator, and any other person(s) deemed necessary.

V. Methods

Complaint: Complaints regarding animals can be presented directly to the IACUC Chair, the AV, the Institutional Official (IO), any member of the IACUC, and/or CMR/Animal Care management. Complaints can be submitted verbally or in writing (hand-written or electronic); anonymous complaints are also accepted. Additional information regarding animal welfare reporting can be found in the IACUC Policy Animal Welfare Concern Reporting (Whistleblower).

Rutgers employees (staff or faculty), IACUC members, or students must not be discriminated against or be subjected to any reprisal for reporting violations of the regulations or standards of the PHS Policy or the Animal Welfare Act or concerns they may have regarding care and use of animals.

Confidentiality: Only the IACUC Chair or Executive Committee will know the identity of the complainant if confidentiality is requested. If the complaint is referred to the IACUC as a whole, the complainant's name will not be included. Information identifying the complainant will be redacted from all documentation forwarded to the IACUC.

Investigation: Two possible mechanisms are used to investigate potential non-compliance.

a. Investigation by IACUC Chair and/or AV: The IACUC chair and/or AV investigates the allegation, including reviewing submitted materials such as emails, photographs or video recordings, interviewing the person(s) allegedly involved, and when possible, the person(s) reporting the concern. When required, relevant records are also examined; copies may be taken. Following the Chair's and/or AV's investigation, a summary will be provided to the Executive Committee.

b. Investigation by IACUC Executive Committee: Typically, the CMR veterinarian assigned to the area in which the non-compliance potentially occurred initiates the investigation. He or she will review any submitted materials such as emails, photographs or video recordings, interview the person(s) allegedly involved, and when possible, the person(s) reporting the concern. When required, relevant records are also examined; copies may be taken. The veterinarian then presents his/her findings to the Executive Committee for deliberation. In most cases of confirmed non-compliance, the PI will meet with the Executive Committee to review and discuss the situation.

Regardless of the mechanism, if the investigation determines potential non-compliance has occurred, it will be presented at a convened IACUC meeting. In some cases, the PI may be asked to attend a portion of the meeting. If a member of the IACUC is involved in the concern, that person will be recused from this portion of the meeting.

Presentation of the event(s) to the IACUC: The presentation to the IACUC is typically given by either the IACUC chair or a representative from the Executive Committee. After discussing the potential non-compliance, the IACUC may request additional information prior to taking action. If no further information is needed:

A. The IACUC will vote on whether the situation constitutes reportable non-compliance and will be reported to the OLAW by the IO. All minority viewpoints will be recorded. The USDA may also be informed, if appropriate.

   Rutgers will report to OLAW for all PHS, NSF, NASA, VA-funded animal activities:

   - Any serious or continuing noncompliance with the Public Health Service (PHS) Policy
   - Any serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals
   - Any suspension of an activity by the IACUC

   Non-compliance associated with research that is not funded by any of the agencies listed above will not be reported to OLAW.

   Rutgers will report to Animal and Plant Health Inspection Service (APHIS) for all USDA covered species:

   - Any significant deficiency not corrected within 14 days
   - Any suspension of an activity by the IACUC

   A preliminary report will initially be submitted prior to the completion of a full investigation and implementation of a corrective plan. After a full investigation a final report will be submitted to OLAW, which includes a detailed explanation of the circumstances and actions taken; the final report will be signed by the Institutional Official. The name of the PI and any associated lab staff members are not

included in the report. If reported to OLAW, USDA, or other appropriate agencies (depending on funding), the non-compliance will also be reported to AAALAC International.

B. Regardless of reporting or funding source, all non-compliance will be treated equally by the IACUC regarding the investigation, review, and resolution.

The IACUC will also determine any actions that are to be taken to prevent the non-compliance from re-occurring. Actions may include, but are not limited to the following:

- Re-training of protocol personnel
- Submission of an amendment to the IACUC-approved protocol
- Requiring a change in procedures previously approved by the IACUC in an animal use protocol or facility standard operating procedure
- Requiring that a previously approved protocol be resubmitted
- Increased oversight via announced or unannounced inspections of laboratory or animal facility work areas by CMR and/or PAM
- **In severe and/or chronic cases of non-compliance, suspension of all or a part of research activity or personnel within an approved protocol**

Actions of the IACUC will be documented as follows:

- The IO will receive a summary of the concerns, and the IACUC actions.
- The IO, through the IACUC Office, will finalize a letter to OLAW concerning all reportable non-compliance, when required. Relevant granting agencies will be informed, if appropriate.
- The IACUC Office will inform the USDA, if appropriate. The AAALAC Program Correspondent will inform AAALAC International, when necessary, and summarize concerns in the annual report.
- If requested, the complainant will receive a summary of the actions taken; any confidential information concerning protocols will be redacted.
- The IACUC Office will maintain the documentation.
- The PI will receive written notification of the IACUC's determination and the corrective actions to be taken.
- The PI must confirm that all corrective actions have been addressed and any necessary amendments have been submitted.

VI. References

- Public Health Services Section III, D. 4. Assurance of Compliance Policy on Humane Care and Use of Laboratory Animals
- NOT-OD-05-034
- NOT-OD-10-081
- The USDA, Animal Plant and Heath Inspection Services, 9 CFR Chapter 1, 1-1-92 Edition. Subchapter A – Animal Welfare (Animal Welfare Act)