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I. **Purpose:** This document establishes guidelines for housing mice and rats maintained in Rutgers University animal facilities as overcrowded cages represent a significant animal welfare concern.

II. **Introduction:** The Public Health Service (PHS) and AAALAC International require that institutions comply with the standards in the *Guide for the Care and Use of Laboratory Animals*. The Institutional Animal Care and Use Committee (IACUC) must comply with the national standards and recommendations contained within the Guide when establishing cage populations. All exceptions to the Guide standards and recommendations need to be described and justified in an IACUC approved protocol prior to initiation.

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).”

Overcrowding of cages is an animal welfare issue and can cause stress, provoke aggression and may contribute to disease.

III. **Responsibilities:** Anyone housing laboratory animals at Rutgers University must comply with this document.

IV. **Definitions**

a. **Mouse ‘shoebox’ cage** – Cage size varies with manufacturer; the most common is ~ 7.5W x 11.75D x 5H (inches), 67in\(^2\)-75in\(^2\) useable floor space depending, on specific cage type.

b. **Rat ‘shoebox’ cage** - Cage size varies with manufacturer; the most common is ~10.1W x 19D x 8H (inches), ~143in\(^2\) useable floor space.

For specific caging sizes in each vivarium, consult facility management.

V. **Methods**

a. **Summary of Caging Density – Mice and Rats**
   i. In general no more than 5 adult mice (~25g each) in a standard mouse cage; this includes animals awaiting euthanasia (in animal holding rooms or placed in the necropsy room).

   ii. No more than 3 adult rats (400g each) in a standard rat cage; this includes animals awaiting euthanasia (in animal holding rooms or placed in the necropsy room).

   iii. No more than one male and female, with one associated litter per cage.
iv. All litters beyond weaning age (21-28 days): Some inbred and transgenic rodent lines produce pups that are too small to be safely weaned at 21 days; these animals are permitted to be weaned at 28 days without an IACUC approved exception.

b. Summary Table - Recommended minimum space for commonly used laboratory rodents housed in groups [the Guide 8th ed., page 62, table 3.2]

<table>
<thead>
<tr>
<th>Animals</th>
<th>Weight (g)</th>
<th>Floor Area/Animal, a  in² (cm²)</th>
<th>Height, b  in (cm)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice in Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>&lt;10</td>
<td>6 (38.7)</td>
<td>5 (12.7)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td></td>
<td>Up to 15</td>
<td>8 (51.6)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 25</td>
<td>12 (77.4)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;25</td>
<td>≥15 (≥96.7)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td>Female + Litter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mouse)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;100</td>
<td>17 (109.6)</td>
<td>7 (17.8)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td></td>
<td>Up to 200</td>
<td>23 (148.35)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 300</td>
<td>29 (187.05)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 400</td>
<td>40 (258.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 500</td>
<td>60 (387.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;500</td>
<td>≥70 (≥451.5)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td>Female + Litter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;100</td>
<td>17 (109.6)</td>
<td>7 (17.8)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td></td>
<td>Up to 200</td>
<td>23 (148.35)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
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<td>Up to 300</td>
<td>29 (187.05)</td>
<td>7 (17.8)</td>
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</tr>
<tr>
<td></td>
<td>Up to 400</td>
<td>40 (258.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 500</td>
<td>60 (387.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;500</td>
<td>≥70 (≥451.5)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
</tbody>
</table>

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.

Cc 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 to weaning without detrimental effects for the mother or the litter.

c. Breeding Animals

The copulatory plug is the first indicator of successful copulation. Females are usually checked in the morning for the presence of a copulatory plug. The plug is only present for 12-24 hours post coitus and the likelihood of pregnancy is high, though not guaranteed. This is the responsibility of the investigative staff, unless prior arrangements have been made with facility staff.

Permitted breeding schemes include:

i. **Monogamous pairing** - 1 resident male: 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 which results in more efficient reproduction and shorter time between litters. Pups should be weaned at 21 days so that newborn pups will not be compromised.

ii. **Trio breeding (mice only) - 1 resident male: 2 females.** We generally discourage trio breeding because if not properly maintained, cages quickly become overcrowded. It is difficult to maintain traditional trio breeding cages using standard shoebox caging (70-75in² cage) and remain within the *Guide* recommendations. Therefore pregnant females should be separated prior to parturition and MUST be separated when the first litter is born.

Another drawback to trio breeding is that it is not always possible to identify which mother has given birth to individual pups, therefore the second female must be removed after the first female gives birth. Trio breeding cannot consist of two males with one female as this can result in aggression and fighting between the males.

While we generally discourage trio breeding for generation of adult animals, it can be acceptable in the following situations:

a. Females will be euthanized before parturition
b. Neonates will be euthanized before postnatal day 7
c. Larger cages are used (check with CMR staff for availability)

These cages must be managed so that animal welfare is not compromised and cages do not become overcrowded.

d. **Overcrowded Cage(s)**

i. Overcrowded cage(s) will be marked with an "Overcrowded Cage" notification card, placed and dated by animal care staff or veterinary staff and the PI (or lab designee) will be notified via an e-mail by the veterinary staff.

Note: Removal of “Overcrowded Cage” notification card(s) by research staff, prior to correction of overcrowding, will be immediately reported to the IACUC as noncompliance.

ii. Overdue cages: If the PI (or lab designee) does not correct overcrowded cage(s) within the 72 hour (3 day) time period (including weekends and holidays), veterinary staff will separate the animals into the appropriate number of cages (e.g. 15 newly weaned mice divided into 3 cages). The researcher will be billed a technical services fee for each cage necessary to satisfy the overcrowding policy.

iii. Severely overcrowded cages (15+ animals) must be separated within 24hrs of notification (or sooner in the interest of animal welfare, based on the discretion of the veterinary staff).

iv. Excessive numbers of overcrowded cages or chronic disregard for this document will be reported to the IACUC as noncompliance.

e. **Single Housing of Social Animals**

The *Guide* states: “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.”
VI. References


Genotyping – Tissue and Blood Collection

I. Purpose: This document describes acceptable tissue collection procedures for genotyping mice (of the genus *Mus*) and rats (of the genus *Rattus*).

II. Introduction: When tissue samples are required for DNA analysis of mice and rats, tissue is usually collected by cutting off the tip of the tail. Alternatives are available and investigators are encouraged to use the least invasive method possible to ensure animal welfare. Note that Polymerase Chain Reaction (PCR) requires less DNA compared to Southern Blot.

If genotyping is necessary, it is regarded as a true refinement if the method of identification also generates a tissue sample that can be used for genotyping (e.g. ear notching or toe clip). This avoids performing repeated invasive procedures on the same animal. Researchers should remove the least amount of tissue necessary to perform genotyping. 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.

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.

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

IV. Definitions (none)

V. Methods
   a. Tools
      i. Scissors, blades, and ear punches must be sharp. Usage of dull or rusted instruments is unacceptable.
      ii. Instruments should be sterilized before use, by one of the following methods:
         1. Soak in sterilant (such as chlorine dioxide solution) for at least 5 minutes
         2. Use hot bead sterilizer (allow instrument to cool before using on the animal)
         3. Autoclave prior to use
         4. Ethylene oxide sterilization
      iii. Clean and disinfect instruments between animals to minimize infection and DNA cross-contamination of the samples.

   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 following methods do not require Institutional Animal Care and Use Committee (IACUC) approval:
i. **Feces** – Collect fresh stool pellets directly from the mouse.

ii. **Hair** – Gently pluck tufts of hair (2 tufts per mouse) 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.

iii. **Buccal/Cheek Swab**
   1. Restrain mouse
   2. Gently swab the inner surfaces of both cheeks with a sterile 2mm cotton-tipped applicator
   3. Cut off top of applicator and insert into a 1.5ml Eppendorf containing 0.6ml of distilled water

The following techniques are required to be included in an approved IACUC protocol:

iv. **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.

v. **Ear Punching or Snipping**
   1. Restrain the animal
   2. 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).
   3. Place ear tissue into a container using forceps
   4. When used for identification, record sequence/coding

vi. **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. Tail biopsies performed after 21 days of age are strongly discouraged, but, if IACUC approved, must be performed using anesthesia and/or post-operative analgesics as per the table below.

   **Background** – The tail of a rodent contains a variety of tissues, including bone, cartilage, blood vessels and nerves. Studies have shown that in young rodents under 17 days of age, the tissue near the tip of the tail is soft and bones have not completely mineralized. Therefore, removing the distal tail tip of rodents under 17 days of age results in a greater yield of DNA per unit tissue and the potential for less pain. It is for this reason that it is strongly recommended to perform tail snips in rodents under 17 days of age. Mineralization of the bone and vascularity increases in this region as the animal ages. In addition, prompt analysis of tail tissue allows genotype to be determined prior to weaning, which can facilitate more efficient use of cage space.

   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. Clean tip of tail with 70% alcohol.
   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 over multiple sampling events, the sum total of tissue taken cannot exceed 5mm.
e. Using sterile forceps, place piece directly into a container (e.g. Eppendorf tube or 96 well plate), and ensure container is labeled with corresponding mouse ID.

f. Hemostasis must be obtained. Apply direct pressure (preferably with gauze) to the tip of the tail for 20-30 seconds, or until bleeding stops.

g. Summary of tail biopsy requirements:

<table>
<thead>
<tr>
<th>age</th>
<th>anesthetic</th>
<th>analgesia</th>
<th>hemostasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤21 days, biopsy ≤5mm</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>22-28 days, biopsy ≤5mm</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>≥29 days, biopsy ≤5mm</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>any age, biopsy ≥5 mm; Requires scientific justification</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

h. General anesthesia - When required, 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.

i. Analgesia – Analgesics should be administered post-procedurally to any animals showing signs of pain or distress. Consult a CMR veterinarian for information on suitable drugs and doses. 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 Anesthesia and Analgesia in Laboratory Animals IACUC Guideline.

vii. Toe 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 allowed in rats up to 7 days of age and mice up to 17 days of age. If animals are over this age limit, toe clipping must be scientifically justified in an approved protocol, and used as both a method of identification and for tissue collection. The best age is between 5-7 days as it appears to have fewer adverse effects on behavior and well-being until this age.

a. Toe clipping may be performed without anesthesia up to 17 days of age (mice) or 7 days of age (rats).

b. Use small, sharp, sterile scissors (such as ocular microsurgical scissors).

c. Cut only one toe from each paw. Therefore, a maximum of 4 toes may be cut. If the mouse is between 8-17 days of age, only 2 total toes may be cut.

d. Remove only the distal phalanx, and remove the entire nail bed to avoid re-growth.
e. Do not cut the hallux (aka dew claw, thumb, little toe of the forepaw) as this may decrease the rodent’s grasping ability.

f. Start with the toes of the hind feet as they are less sensitive than the front toes.

g. Toe clipping in animals older than 17 days is prohibited without additional scientific justification in an approved IACUC protocol.

h. Procedure:
   i. Wipe down the skin of the foot and digits with alcohol.
   ii. Gently restrain the rodent.
   iii. Extend the leg and remove distal phalanx.
   iv. 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. References


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 Institutional Animal Care and Use Committee (IACUC) protocol. For information on additional methods of euthanasia not covered in this document, consult with the Comparative Medicine Resources (CMR) veterinary staff.

III. **Responsibilities**: Any individual performing euthanasia of laboratory animals at Rutgers University must comply with the methods outlined in this document. Personnel performing euthanasia must be trained and proficient in the specific procedure(s) prior to working with animals.

IV. **Definitions**:
   a. IV – intravenous
   b. IP – intraperitoneal
   c. IC – intracardiac
   d. PI – principal investigator

V. **Overview of Methods**
   a. **Inhalation**
      i. Carbon Dioxide (CO₂) – see section VI for more details
      ii. Noble Gas (i.e. Argon)
      iii. Anesthetic Overdose
   b. **Non-inhalation**
      i. **Injectable** – see section VI for more details
         1. Sodium Pentobarbital
         2. Anesthetic Overdose
      ii. **Immersion**
         1. Tricaine Methanesulfonate (MS-222) – see section VI for more details
   c. **Physical**
      i. Cervical Dislocation
      ii. Decapitation – see section VI for more details
      iii. Exsanguination/Perfusion
      iv. Captive Bolt
      v. **Pithing** – see section VI for more details

VI. **Procedures**
   a. **Carbon Dioxide (CO₂)** - CO₂ asphyxiation is an approved method of euthanasia for rodents, but must be performed properly to be effective and humane. These procedures are for adult animals only.
Neonatal rodents are very resistant to CO₂ euthanasia and special procedures are required in order to euthanize them (see point 11 below).

The use of CO₂ must be followed with one of the following:
- Physical method such as cervical dislocation, decapitation, thoracotomy
- Prolonged observation – animals must be observed in the euthanasia chamber for a minimum of three minutes after cessation of respiration followed by an additional period of 5 minutes after exposure to room air

The following procedures are designed to assure that CO₂ euthanasia is performed properly:

1. It is preferable that animals be exposed to CO₂ in their home cage. If use of the home cage is not feasible place the animals in a secondary container that is easily sanitized.
2. Animals 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 10% to 30% of the chamber volume/min is recommended.¹
5. Do not pre-fill the chamber prior to the addition of animals. Empty the chamber before adding additional animals.
6. CO₂ is heavier than air. Excess gas must be allowed to exit from the top of the chamber.
7. Use a transparent euthanasia chamber and/or lid. You must be able to see animals without opening the chamber.
8. Euthanasia chambers must be kept clean and free of debris, urine and feces.
9. Do not leave animals undergoing CO₂ euthanasia unattended, until death is assured.
10. Dry ice is not an acceptable means of producing CO₂ gas. The CO₂ source must be a compressed gas cylinder. 100% CO₂ is recommended.
11. Compressed air tanks must be equipped with a pressure regulator and a flow meter.
12. CO₂ is not a practical method of euthanasia in neonates up to 9 days of age. A direct physical method such as decapitation is recommended. If CO₂ must be used to euthanize neonates, the time to narcosis is lengthy (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.

**CO₂ euthanasia quick reference chart:**

<table>
<thead>
<tr>
<th>Age</th>
<th>Accepted</th>
<th>Not accepted</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos (E-1-E21)</td>
<td>death of dam is sufficient, no other methods are needed</td>
<td>N/A</td>
<td>dam needs to be euthanized using accepted methods</td>
</tr>
<tr>
<td>Neonates (P1-P9)</td>
<td>decapitation, cervical dislocation, chemical injection, CO₂ inhalation (with conditions)</td>
<td>hypothermia</td>
<td>animals are resistant to hypoxia at this age, CO₂ inhalation requires prolonged exposure (50 minutes) and a secondary physical method</td>
</tr>
</tbody>
</table>
### Table: Gradual Exposure to CO₂

<table>
<thead>
<tr>
<th>P10 to adult</th>
<th>Gradual exposure to CO₂, anesthetic overdose (chemical injection, inhalation)</th>
<th>CO₂ from dry ice, hypothermia, smothering, blow to head</th>
<th>Inhalation method must be followed by a physical euthanasia method</th>
</tr>
</thead>
</table>

#### b. Anesthetic overdose (inhalation)

i. Inhaled anesthetic overdose with isoflurane gas can be used for the euthanasia of laboratory rodents. With isoflurane, anesthetic concentrations can be rapidly achieved and maintained, but an increased concentration of isoflurane may be necessary to adequately euthanize a rodent. 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 isoflurane gas, so time to death may be prolonged. When using the closed receptacle with liquid isoflurane, 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.

ii. It is necessary to confirm that death has been achieved with this method of euthanasia, either by performing a secondary physical method such as decapitation or cervical dislocation, or by physical examination of cessation of respiration through prolonged observation. Prolonged observation requires that the rodent be observed while actively exposed to isoflurane for a minimum of 3 minutes after cessation of respiration, followed by an additional 5 minutes of observation in room air containing no anesthetic gasses.

iii. Effective engineering controls should be in place to reduce animal worker exposure to volatile anesthetic vapors. Personnel utilizing a concentrated liquid isoflurane euthanasia method must do so within the confines of a ducted biosafety cabinet (Class II B2) or ducted chemical fume hood.

#### c. Injectable agents

i. **Barbiturates** – Sodium pentobarbital is the most commonly used barbiturate. The minimum dose is 100 mg/kg; it is advisable to use a dose that is 3 times the recommended anesthetic dose for rodents. It can be delivered IV, IP, or IC. Please contact CMR Veterinarians for further recommendations for non-rodent species.

ii. **Potassium Chloride** – Must be administered IV using a saturated solution and only when animals are under general anesthesia (1-2mEq K+/kg or 75-150 mg/kg). Please contact CMR Veterinarians for further recommendations for use.

#### d. Tricaine Methanesulfonate (MS-222)

i. 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 much higher doses, MS-222 is also commonly used for euthanasia of aquatic species.

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

iii. Due to the acidity of pharmaceutical grade MS-222, solution must be buffered for all intended uses in live vertebrate aquatic species. A 10 g/L of stock solution can be made and sodium bicarbonate added to saturation resulting in a pH of 7.0 to 7.5 for the solution.

iv. Fish: Immerse fish in a minimum dose of 250-500mg/l. Fish should remain in solution at least 10 minutes following cessation of opercular movements. Again, MS-222 must be buffered with sodium bicarbonate. For zebrafish, rapid cooling euthanasia is preferred to MS-222 immersion (see section f for more details).
v. Aquatic frogs: Immerse frog in an overdose concentration; concentrations exceeding 3gm/liter constitutes overdoses for most species; recommended dose is 5-10gm/liter. MS-222 must be buffered. Prolonged immersion (up to 1hr) may be required. This must be followed by an adjunctive method of euthanasia; either pithing or decapitation. See section e for more information.

e. Decapitation (guillotine, scissors, scalpel blade, etc.) Euthanasia 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, is permitted when scientifically justified by the user in the protocol review form and approved by the IACUC. For reptiles and amphibians, decapitation should be preceded by anesthesia and followed by pithing (see point e below).

The individual performing euthanasia by decapitation must demonstrate technical competency in the procedure to a veterinarian as a condition of approval. 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.

- Place the animal’s neck in the guillotine or other cutting device.
- 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.

Note: The guillotine or scissors should be cleaned during use to keep blades clear of hair and tissue, which may affect cutting ability; clean thoroughly after use. Decapitation equipment must be sharpened regularly. Blades may be sent to the following company for sharpening:

Repairs Department, George Tiemann & Company
25 Plant Avenue Hauppauge, NY 11788
Tel # (800) 843-6266 or (516) 273-0005, Fax # (516) 273-6199

Keep a log of routine checks for sharpness and maintenance for each guillotine. Logs should be available for review during IACUC semiannual inspections. In the case where sending guillotines to a company for sharpening is not feasible, blades must be replaced annually.

f. Pithing following general anesthesia induction is an acceptable method of euthanasia for 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. It may be upsetting to some students and if it is not the object of a lesson to teach the actual procedure, it may be better to pith animals when students are not present.

Pithing alone not an acceptable primary method of euthanasia. Pithing should not be used for *Xenopus* spp. (African Clawed Frogs) as it is difficult to bend the head forward to expose the atlanto-occipital space; *Xenopus* should be decapitated.
**Recommended Procedures:**
Frogs must be fully anesthetized prior to pithing. All anesthetics used must be described in the approved protocol.

**Double pithing**
- Hold the frog facing away from your body, with the lower extremities extended.
- Grasp the frog with your first two fingers: first finger on the nose, second finger under the jaw.
- Flex the head forward (away from your body).
- 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.
- Insert the probe quickly into the cranial vault and sever the brain and spinal cord.
- Move the probe into the cranial vault and move it from side to side to destroy the brain.
- Keep the probe in the cranial canal and turn the probe around into the vertebral canal (do this without removing the probe) and insert the probe into the full length of the vertebral canal to destroy the spinal cord.

**Decerebration and spinal pithing**
- Remove the cerebral hemispheres by inserting an open pair of scissors into the frog’s mouth and sever the head behind the eyes.
- Take a probe and insert it into the full length of the vertebral canal to destroy the spinal cord.

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

**VII. References**
- AVMA Guidelines on Euthanasia, 2013
- 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, 8th ed, NRC Press, 2011
I. Purpose: The purpose of this document is to provide investigators with guidelines for conducting acceptable methods of rodent surgical technique. In addition, these guidelines are to be followed for surgeries on birds, amphibians, reptiles, and fish.

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. Students who learn good rodent surgical technique will be able to apply their knowledge of specific procedures to other procedures in other species. Surgery performed without proper anesthesia, monitoring and post-operative care can result in poor research data, unnecessary use of animals, and animal suffering.

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 patterns.

III. Responsibilities
All individuals performing rodent 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
a. Asepsis - The state of being free of living pathogenic microorganisms.

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

c. 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.

d. Disinfection – The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

e. 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.

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

h. **Sterile** - Free from all microorganisms.

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

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 will be 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 can be 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 a case-by-case basis surgeries may be required to be observed by a Comparative Medicine Resources (CMR) veterinarian. For some protocols, the first surgery after initial approval must be observed by a veterinarian. Notification of individual observations will be communicated at the time the approval notice is issued.

b. **Anesthetics, Analgesics, and Tranquilizers**

   “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. Choose agents depending on the type of procedure, the length of time it will take, the equipment available, and the skill of the individuals performing the procedure. 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.
Commonly used anesthetic agents for rats and mice include pentobarbital, ketamine/xylazine combination, and isoflurane. For more information on these and other drugs, consult the IACUC document *Anesthesia and Analgesia in Laboratory Animals*.

Minor surgery in rodents may result in postoperative pain. 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.

c. 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 Table 1: “Recommended Hard Surface Disinfectants” (end of document)*

- Provide adequate lighting

- Provide a heat source e.g. recirculating warm water blanket, covered heating pad (on low setting), warm water bottle/glove, hand warmer (for small rodents), or heat lamp to prevent hypothermia of the animal.

d. 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 will not require 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.

i. 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.

<table>
<thead>
<tr>
<th>Acceptable methods of sterilization:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Steam autoclave</td>
</tr>
<tr>
<td>• Gas sterilization (ethylene oxide)</td>
</tr>
<tr>
<td>• Dry heat (e.g. glass bead sterilizer)</td>
</tr>
<tr>
<td>• Gamma irradiation</td>
</tr>
<tr>
<td>• 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</td>
</tr>
<tr>
<td>• Chemical sterilants with adequate contact time (e.g. “Cidex” glutaraldehyde – see Table 2 for details)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unacceptable methods of sterilization:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Non-sporidal disinfectants (e.g. alcohol)</td>
</tr>
<tr>
<td>• See Table 2 “Recommended Instrument Sterilants”</td>
</tr>
</tbody>
</table>

j. Implantable materials (cannulas, ports, pumps, biocompatible matrices, etc.)

**Implants** – The number and size of implants shall 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.

ii. Animal Preparation

1. 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.

Withholding of food (not water) for at least four hours before surgery may promote smoother induction of anesthesia and increased absorption of intraperitoneally administered anesthetics. Discuss preoperative fasting with a CMR veterinarian; this must be previously approved by the IACUC.

2. Animal Preparation

Prepare the surgical site in an area 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.

Remove hair around the surgical site by:
   a. Using a surgical clipper with a #40 blade (clip against the grain)
   b. Application of a depilatory cream

Clip or depilate a large enough area so that hair does not protrude from under the drapes into the surgical field.

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 use of an effective disinfectant e.g. povidone-iodine (sudsing) soap (e.g. Betadine®) or chlorhexidine (e.g. Nolvasan™), 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.

See Table 4: “Skin Disinfectants”

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

<table>
<thead>
<tr>
<th>Minimal Animal Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Clip hair with an animal clipper with a #40 blade</td>
</tr>
<tr>
<td>• Scrub the skin with surgical soap (povidone-iodine or chlorhexidine scrub) at least 3 times, alternating with alcohol</td>
</tr>
<tr>
<td>• Apply ophthalmic ointment</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Supplemental Animal Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Surgical drape</td>
</tr>
</tbody>
</table>
iii. Sterile Field

Two steps that improve aseptic technique are 1) a surgical drape on the animal, and 2) the provision of a sterile surface to put the instruments on when not in use. A sterile surgical 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 the use of 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. Contact CMR Veterinarians for more information.

A sterile surface for placing instruments between use can be: (a) the edge of the 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.

iv. 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” below

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

v. 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.
- 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 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.

e. Operative Period

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. Heat loss can be minimized by housing the animal in a warm room. 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 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.

Dehydration - In prolonged or very invasive surgeries, give 0.5-1.0cc (mice) or 10cc (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.

Skin Closure - There are several choices of methods of skin closure – nonabsorbable or absorbable suture material, wound clips or staples, and 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 7-14 days post-procedure).

i. 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.

Advantages of the Tips Only technique are that sterile gloves are not required. Then 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.

1. 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.
2. Sterilize all instruments and supplies in advance.
3. 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.
4. Handle instruments only by the handles.
5. Do not touch sterile tips with your hands. Do not allow tips to touch non-sterile surfaces.
6. Handle sutures, catheters and other material only with instrument tips.
7. Handle tissues only with instrument tips. Do not touch tissues with your hands.
8. After use, place instruments on the line on the drape with tips on the sterile side.
9. 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.
10. 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.
11. Use two sets of instruments and alternate sets between animals (optional).
12. 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.
13. Clean instruments before heating. Allow tips to cool before use.

ii. 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.
f. 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 (a) observing the animal to ensure uneventful recovery from anesthesia and surgery; (b) administering supportive fluids, analgesics, and other drugs as required; (c) providing adequate care for surgical incisions; and maintaining appropriate medical records.

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.

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. Administer narcotic analgesics for at least the first 24 hours following any major surgical procedure. Minor procedures may only require one dose. Provide analgesics until no signs of pain are present.

Monitor:
  a. Incisions for swelling, exudates, pain or dehiscence (wound rupture or opening)
  b. Catheters and any other attached devices
  c. For post-procedure related complications such as organ failure, thrombosis and ischemia.

Remove skin closures (sutures, wound clips) 7-14 days post-operatively.

Surgical Records can be part of the regular research notebook and must be available for review by veterinary staff and the IACUC. Records for rodent surgery need to 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.

Surgery card/post-op care card - mark each cage with a surgery card which indicates 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 home and work phone. Cards must be requested from CMR facility supervisors in advance.
After the initial recovery observe animals at least daily with special attention to the appearance of the surgical site, attitude, alimentation and 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.

Properly performed aseptic surgery may 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. Please note that some antibiotics are extremely toxic to certain species or strains of rodents such as hamsters and guinea pigs. Report all clinical infections to a CMR veterinarian so that specific, individual guidance may be obtained. Please note that antibiotics should be on the IACUC-approved protocol, or prescribed by CMR veterinary staff before administration.

g. 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.

h. Deviations from this Policy

Deviations from this policy must be approved by the Institutional Animal Care and Use Committee as part of an approved animal use protocol. Changes requested after approval must be requested as an amendment to the protocol.

VI. References


### Table 1. Recommended Hard Surface Disinfectants (e.g. table tops, equipment) - Always follow manufacturer's instructions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>70% ethyl alcohol 70%-99% isopropyl alcohol</td>
<td>Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable.</td>
</tr>
<tr>
<td>Quarternary Ammonium</td>
<td>Roccal®, Cetylcide®</td>
<td>Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®)</td>
<td>Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (&lt;14 Days old); kills vegetative organisms within 3 minutes of contact. A rinse with water or alcohol is required after solid surface disinfection.</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Gluteraldehyde (Cidex®, Cide Wipes®)</td>
<td>Rapidly disinfects surfaces. Toxic exposure limits have been set by OSHA.</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Lysol®, TBQ®</td>
<td>Less affected by organic material than other disinfectants.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.</td>
</tr>
</tbody>
</table>

### Table 2. Recommended Instrument Sterilants

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical: Steam sterilization (moist heat)</td>
<td>Autoclave</td>
<td>Effectiveness dependent upon temperature, pressure and time (e.g. 121°C for 15 min vs. 131°C for 3 min).</td>
</tr>
<tr>
<td>Dry Heat¹</td>
<td>Hot Bead Sterilizer Dry Chamber</td>
<td>Fast. Instruments must be cooled before contacting tissue.</td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>Gamma Radiation</td>
<td>Requires special equipment</td>
</tr>
<tr>
<td>Chemical: Gas Sterilization</td>
<td>Ethylene Oxide</td>
<td>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.</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>(Sterad®)</td>
<td>Not useful for &quot;delicate&quot; items.</td>
</tr>
<tr>
<td>Chlorine²</td>
<td>Chlorine Dioxide (Clidox®, Alcide®)</td>
<td>A minimum of 6 hours required for sterilization. Presence of organic matter reduces activity. must be freshly made (&lt;14 days)</td>
</tr>
<tr>
<td>Aldehydes²</td>
<td>Formaldehyde (2% sol.) Glutaraldehyde</td>
<td>For all aldehydes: many hours required for sterilization. Corrosive and irritating. Consult safety representative on proper use. Glutaraldehyde is less irritating and less corrosive than formaldehyde.</td>
</tr>
</tbody>
</table>

¹Instruments must be cleaned and rinsed before being placed in the bead sterilizer.
²Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used.
Table 3. Recommended Instrument Disinfectants

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol 70%-99% isopropyl alcohol</td>
<td>NOT ADEQUATE FOR PRIMARY INSTRUMENT STERILIZATION. Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable. Low level disinfectant.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine¹</td>
<td>Sodium hypochlorite (Clorox®, 10% solution) Chlorine dioxide (Clidox®, Alcide®)</td>
<td>Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (&lt;14 Days old); kills vegetative organisms within 3 minutes of contact.</td>
</tr>
<tr>
<td>Peracetic Acid/Hydrogen Peroxide</td>
<td>Spor - Klenz®</td>
<td>Corrosive to instrument surfaces. Must be thoroughly rinsed from instruments before use.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.</td>
</tr>
</tbody>
</table>

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

Table 4. Skin Antiseptics

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol 70-99% isopropyl alcohol</td>
<td>NOT ADEQUATE FOR SKIN PREPARATION when used as a sole surgical disinfectant!. Not a high level disinfectant. Not a sterilant. Flammable.</td>
</tr>
<tr>
<td>Iodophors e.g. povidone iodine</td>
<td>Betadine®, Prepodyne®, Wescodyne®</td>
<td>Reduced activity in presence of organic matter. Wide range of microbe killing action. Works best at pH 6-7.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.</td>
</tr>
<tr>
<td>Suture</td>
<td>Characteristics and common applications</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Vicryl®, Dexon®</td>
<td>Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.</td>
<td></td>
</tr>
<tr>
<td>PDS® or Maxon®</td>
<td>Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable</td>
<td></td>
</tr>
<tr>
<td>Prolene®</td>
<td>Nonabsorbable. Inert.</td>
<td></td>
</tr>
<tr>
<td>Nylon</td>
<td>Nonabsorbable. Inert. Recommended for skin.</td>
<td></td>
</tr>
<tr>
<td>Silk</td>
<td>Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Silk is very easy to use and knot. <strong>Silk is not acceptable for suturing skin.</strong></td>
<td></td>
</tr>
<tr>
<td>Chromic Gut</td>
<td>Absorbable. Versatile material. Causes mild inflammation, but is absorbed more rapidly than synthetics. <strong>Chromic gut is not acceptable for suturing skin.</strong></td>
<td></td>
</tr>
<tr>
<td>Stainless Steel: Wound Clips, Staples</td>
<td>Nonabsorbable. Requires instrument for removal from skin.</td>
<td></td>
</tr>
</tbody>
</table>
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. Additionally, signs of pain/distress are reviewed in order to determine the appropriate dose(s) of the various drugs.

II. **Introduction**

a. 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.

b. Although animals that are in pain may not behave like humans, (e.g., pain in animals may be accompanied by immobility and silence, in contrast to the groans and cries of human patients), it is assumed that procedures that cause pain in humans will cause pain in animals.

c. The presence of pain in animals can be recognized by alterations in animal behavior (e.g., reduced activity, reduced grooming, hunched-up posture, altered gait, changes in temperament, vocalizations, reduced food and water intake, reduced urinary and fecal output), and in physiological variables, (e.g., reduced depth of respiration, increased heart rate, and reduced hydration status) (refer to Table I).

d. Animal pain, distress, and discomfort can produce a range of undesirable physiological changes, which may radically alter measured responses to experimental stimuli, as well as the rate of recovery from a surgical procedure. Therefore its avoidance and alleviation are in the best interest of both the animal and researcher.

e. Reducing post-procedural/post-operative pain, distress, and discomfort is accomplished by good nursing care, (e.g., keeping the animal warm, clean, dry and well padded), and by the administration of analgesic drugs.

f. The selection of an appropriate analgesic involves consideration of the level of animal pain anticipated or presumed, the species involved, and the experimental protocol. Severe pain, such as may occur during the post-operative period, can be alleviated by the administration of narcotic analgesics, (e.g., buprenorphine, an opioid partial agonist). Non-steroidal anti-inflammatory drugs, with or without the infusion of local anesthetics, can control mild to moderate pain in some species, though is contra-indicated in others. Selection of an appropriate route of administration also
involves consideration of the recipient species. For example, oral analgesic drug delivery to rodents (e.g. acetaminophen elixir added to the drinking water, carprofen tablets placed in a cage) may not afford detectable analgesia. Rodents are neophobic, and may not readily ingest new substances. Furthermore, animals in pain may not readily consume food or water at the rate that would provide therapeutic analgesic efficacy. Therefore, it is important to dose in advance of surgery if using oral substances. Please contact CMR veterinary staff for further recommendations.

g. In addition to the avoidance and alleviation of pain and discomfort, adequate post-procedural/post-operative animal care also includes efforts to prevent and/or treat post-anesthetic complications, (e.g., aspiration, hypostatic pneumonia, cardiovascular and respiratory depression, dehydration, and infection).

h. Reducing the potential for laboratory animal pain, distress, or discomfort is required by the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, the Guide for the Care and Use of Laboratory Animals (2011 ed.), and the Animal Welfare Act (Public Law 89-544).

i. The prevention or minimization of animal pain, distress, or discomfort by the proper use of tranquilizers, anesthetics, and analgesics is scientifically and ethically essential to the humane care, use, and treatment of research animals. The use of these classes of drugs must effectively prevent or minimize suffering and discomfort of animals during potentially painful procedures.

j. The use of these three classes of drugs must be in accordance with currently accepted veterinary medical practice and produce in the subject animal an appropriate level of tranquilization, anesthesia, or analgesia consistent with the protocol or design of the experiment.

k. These guidelines are provided as a resource for research faculty using animals in research, teaching, or testing.

l. **Always consult with CMR laboratory animal veterinarians** whenever designing a research, teaching or testing protocol that involves animals.

**III. Responsibilities:** This document applies to anyone at Rutgers University who may be inducing a potentially painful or distressful event or events in laboratory animal species in the capacity of research or training.

**IV. Definitions**

a. **Neuroleptic** - produces central nervous depression, depression of excitability of the autonomic nervous system, a dulling of consciousness and a reduction of spontaneous motor activity (e.g., tranquilizers/sedatives)

b. **Analgesia** - relief from pain

c. **Preemptive analgesia** - managing pain before it begins
d. **Tranquilization** - a state of behavioral change in which the patient is relaxed, unconcerned by its surroundings, and often indifferent to minor pain

e. **Sedation** - a mild degree of central depression in which the patient is awake but calm; larger doses of sedative may lead to narcosis

f. **Narcosis** - a drug-induced state of sedation in which the patient is oblivious to pain

g. **Local anesthesia** - loss of sensation in a limited body area

h. **Regional anesthesia** – loss of sensation in a larger, though limited, body area

i. **Basal anesthesia** - a light level of general anesthesia usually produced by preanesthetic agents; serves as a basis for deeper anesthesia following the administration of other agents

j. **General anesthesia** - complete unconsciousness

k. **Surgical anesthesia** - unconsciousness, accompanied by muscular relaxation to such a degree that surgery can be performed painlessly

l. **Multimodal analgesia** – providing more than one class of analgesic (i.e. Opioids and NSAIDs)

m. **Neuroleptanalgesia** - a state of central nervous system depression and analgesia usually produced by a combination of a neuroleptic and a narcotic analgesic

V. **Methods**

a. **General Considerations**

i. 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.

ii. Acclimatizing animals allows them to adjust physiologically and psychologically to their new environment and provides the opportunity to carefully monitor for any abnormalities. **Animals should be acclimatized to the facility for a minimum of 3 days.** Acclimation period may vary depending on species.

iii. When planning to administer drugs, recall that **dosage charts** for anesthetic and analgesic agents state only the **average 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.

iv. 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
   i. 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 and Institutional Animal Care and Use Committee (IACUC) Principle and Procedure XIV.

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

   iii. The following is a partial list of controlled substances that are used in laboratory animals based on DEA schedule:

      1. **Schedule I** – A drug or other substance that has a high potential for abuse, and no currently accepted medical use in treatment in the United States.

      2. **Schedule II** – A drug or other substance that has a high potential for abuse, and a currently accepted medical use in treatment in the United States or a currently accepted medical use with severe restrictions. Abuse of these drugs or other substances may lead to severe psychological or physical dependence (e.g., Codeine, Fentanyl, Meperidine, Morphine, Pentobarbital).

      3. **Schedule III** – A drug or other substance that has a potential for abuse less than those drugs or substances in schedules I and II, and has a currently accepted medical use in treatment in the United States. Abuse of these drugs or substances may lead to moderate or low physical dependence or high psychological dependence (e.g., Ketamine, Thiopental, Telazol or Tiletamine + Zolazepam, Buprenorphine).

      4. **Schedule IV** – A drug or other substance that has a low potential for abuse relative to the drugs or substances in schedules I-III, and has a currently accepted medical use in treatment in the United States. Abuse of the drug or other substance may lead to limited physical dependence or psychological dependence (e.g., Butorphanol, Diazepam, Pentazocine).

      5. **Schedule V** - A drug or other substance that has a low potential for abuse relative to the drugs or substances in schedule IV, and has a currently accepted medical use in treatment in the United States. Abuse of the drug or other substance may lead to limited physical dependence or psychological dependence relative to the drug or other substance in schedule IV.

c. Pre-Anesthetic Treatments
   i. 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. It has been suggested that fasting rodents for several hours prior to general anesthesia may allow for a more even induction.
ii. All animals should be weighed to ensure accurate drug dosage calculations.

iii. 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.

iv. 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.

v. Anticholinergics (e.g., atropine sulfate, glycopyrrolate) block parasympathetic impulses to the cardiopulmonary system, glands, and smooth muscle. Consequently, they prevent vaso-vagal reflexes, slow the heart (bradycardia), and reduce salivary gland and bronchial secretions. The effectiveness of atropine varies among species, especially rabbits. Some rabbits (up to 50%) may produce atropinesterase that rapidly degrades atropine. Therefore, glycopyrrolate, which is less affected by atropinesterase, is recommended for use in rabbits.

vi. Tranquilizers and sedatives (e.g., the phenothiazine tranquilizer acepromazine, the butyrophenone tranquilizers droperidol, or azaperone, and the benzodiazepine sedatives diazepam, or zolazepam) do not produce analgesia. Tranquilizers produce a calming effect, facilitate handling, and reduce the amount of anesthetic required for induction and maintenance of anesthesia without producing a loss of consciousness. Tranquilizers may enhance anesthetic recovery when used with analgesics. Tranquilizers can provide additional skeletal muscle relaxation when that which is produced by the anesthetic is not adequate. Combining acepromazine and ketamine produces muscle relaxation in cats and rabbits, but not surgical anesthesia. Sedatives produce mild central nervous system depression and reduce fear and apprehension without loss of consciousness. They produce good muscle relaxation. Like tranquilizers, sedatives reduce the amount of anesthetic required and enhance anesthetic recovery when used with analgesics. Loud noises can counteract the calming effect of these drugs. Sufficient time should be allowed for these drugs to attain their maximum effect before inducing anesthesia.

vii. Alpha2-adrenergic agonists (e.g., xylazine, dexmedetomidine) mediate analgesia, anxiolysis, sedation, sympatholysis, and control of hypertension. These drugs are usually classified as sedative-analgesics and skeletal muscle relaxants. There is wide species variation in the reaction to these drugs. Xylazine sedation may be reversed using yohimbine, tolazoline, or idazoxan. Atipamezole is a highly selective and potent α2-antagonist that rapidly reverses sedation as well as other behavioral and physiologic effects of dexmedetomidine. Xylazine is often used in combination with ketamine to produce anesthesia in laboratory animals.

d. General Anesthetics

i. 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 he/she and his/her staff are trained in the proper use of tranquilizers, anesthetics, and analgesics appropriate for the species and planned procedures.

ii. 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.

iii. 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. This 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 depressant effect on the cardiovascular, respiratory, and thermo-regulatory systems. Their use must be monitored closely.

iv. 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 and age. 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 giving the anesthetic insufficient time to work. 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.

v. Inhaled Anesthetics (e.g. isoflurane and sevoflurane; 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 recovery.

vi. Intubation is the recommended method for administering inhalant agents, although inhaled anesthetics can be administered by mask. Both provide a constant, high concentration of O₂ to the patient. Intubation allows rapid response to hypoventilation or respiratory arrest through mechanical ventilation using the anesthetic machine.

vii. 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.

viii. Injected Anesthetics (e.g., the barbiturates pentobarbital or thiopental, or the dissociative anesthetics ketamine or tiletamine) 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 that is approximately 20% O₂. Hence, patient animal responses
to respiratory emergencies are delayed. Despite these drawbacks injectable anesthetics are safe and effective to use in many situations. (Note: Ketamine should be used in combination with xylazine or diazepam to produce surgical anesthesia.)

e. Neuromuscular Blocking Agents

i. 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.

ii. 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

i. General anesthesia always carries the risk of compromising the patient’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, 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 a patient. All parameters must be considered in combination to determine the animal’s response to anesthesia.

ii. 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.

iii. 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.
iv. **Cardiovascular Signs** – It is often difficult to manually monitor heart rate in rodents, however the use of monitoring equipment such as the ECGenie© may facilitate monitoring. An increase in rate (tachycardia) during the performance of a surgical procedure often indicates that the depth of anesthesia is not adequate. A decrease of rate (bradycardia) during surgery 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.

v. **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. To avoid thermal burns, water heating pads rather than electrical pads, should be used.

vi. **Post-operatively** – The anesthetist’s responsibility for the animal’s welfare extends beyond the completion of the surgical procedure. 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 prehend food and water. 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.

vii. **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 O₂ and high blood CO₂ 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 O₂.
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.

viii. **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**
   i. 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. 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.

   ii. **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. Analgesics are broadly classified into two groups - the opioids, and non-steroidal anti-inflammatory drugs (NSAIDS).

   iii. **NSAIDS** (e.g., aspirin, 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 (>3 days) 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.

   iv. **Opioids** (e.g., morphine, oxymorphone, meperidine, butorphanol, buprenorphine, pentazocine, fentanyl) 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.

h. **Comments Regarding Anesthetics and Analgesics**
   i. 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 in the Comparative Medicine library.

ii. **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.

iii. **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 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, but the patch should not be cut in half. Instead, cover ½ of the gel membrane with tape. The patch is generally placed on the dorsal cervical area, or over the shoulders. 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 heating pad.

iv. **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. Ketamine should be administered in combination with xylazine or diazepam/midazolam to induce surgical anesthesia.

v. **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 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 bupivicaine 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, to which it is chemically related - approx. 6-8 hours as opposed to 1-2 hours for lidocaine. Duration of action is affected by the concentration of bupivacaine used and the volume injected. Concentration affects the time for local anesthesia to occur and the density of the block. Volume determines the area that is infiltrated and therefore anesthetized. Total dose in mg/kg is important in local anesthetic toxicity. 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, humans), 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 (1:200,000). Epinephrine reduces cutaneous blood flow and therefore prolongs the local anesthetic effects. Maximum concentration of bupivacaine recommended for subcutaneous use is 0.25%. Higher concentrations are used mostly for caudal and epidural blocks in human medicine.

Bupivacaine use: Dose rates from several sources recommend an upper limit of 2-3mg/kg for dogs and rats, 2mg/kg for rabbits. However, if using undiluted bupivacaine 0.25% (2.5mg/ml) it may not be possible to inject a large enough volume to have adequate local anesthetic effects without approaching toxic levels, especially in rodents and rabbits.

Therefore, CMR recommends the following:

- Use a maximum dose of 2.0-2.5 mg/kg body weight. Dilution with saline may be required.
- **Before making the surgical incision**, subcutaneously inject very 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.
- Continued analgesia can be maintained by repeating injections of bupivacaine (as described above) around the incision at 6-8 hourly intervals.
- For rodent surgical procedures such as ovariectomies or stereotaxic cranial surgery, a single injection of bupivacaine prior to making the first surgical incision may be sufficient analgesia. As always, each animal should be monitored for evidence of post-operative pain and additional pain relief provided when needed.

**vi. 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. To reduce the likelihood of this occurrence, pentobarbital can be administered intravenously. Calculate the required pentobarbital volume based on a mg/kg dose and draw this volume into a syringe; administer approximately half of the volume by rapid IV injection to achieve basal narcosis, and then slowly inject additional incremental volumes until surgical anesthesia is achieved. The IP route of administration should be used in rodents only.

**vii. 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).
viii. **Urethane** can be mutagenic and carcinogenic, and its use is strongly discouraged unless strict precautions are taken to protect personnel, its use is limited to non-survival procedures, and its use is justified in writing to, and approved by the IACUC.

ix. **Volatile anesthetics** include isoflurane and sevoflurane. These agents should be used only with adequate ventilation or scavenging systems. Precision vaporizers should be used for these anesthetic agents because lethal concentrations can easily be reached using the open drop method, or using a bell jar as an anesthetic chamber.

x. **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 commonly used for pre-anesthesia, anesthesia, analgesia, sedation, tranquilization, and restraint of laboratory animal species are provided as a reference only, for use by Rutgers University faculty and staff (see below).

   i. 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.

**Signs of Pain and Distress in Laboratory Animals***

<table>
<thead>
<tr>
<th>Species</th>
<th>Signs of mild to moderate pain of distress</th>
<th>Signs of severe or chronic pain or distress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Eyelids partially closed; changes in respiration; rough hair coat; increased vibrissae movement; unusually apprehensive or aggressive; possible writhing, scratching, biting, self-mutilation; hunched posture; sudden running; aggressive vocalization; guarding.</td>
<td>Weight loss; dehydration; incontinence; soiled hair coat; eyes sunken, lids closed; wasting of muscles on back; sunken or distended abdomen; decreased vibrissae movement; unresponsive; separates from group; hunched posture; ataxia; circling; hypothermia; decreased vocalization.</td>
</tr>
<tr>
<td>Rat</td>
<td>Eyelids partially closed; porphyrin staining around eyes, nose; rough hair coat ± hair loss; increased aggression; reduced exploratory behavior; aggressive vocalization; licking, biting, scratching; guarding.</td>
<td>Eyes closed; poor skin tone; muscle wasting along back; dehydration; weight loss; incontinence; soiled hair coat; depressed/ unresponsive; sunken or distended abdomen; self-mutilation; recumbent position with head tucked into abdomen; decreased vocalization; hypothermia.</td>
</tr>
<tr>
<td>Syrian hamster</td>
<td>Ocular discharge; increased aggression; hunched posture; reluctance to move.</td>
<td>Loss of coat and body condition; increasing depression; extended daytime sleep periods; lateral recumbency; hypothermia; sores on lips, paws.</td>
</tr>
<tr>
<td>Gerbil</td>
<td>Ocular discharge; eyelids partially closed and matted with dry material; may &quot;faint&quot; when handled; changes in activity and burrowing behavior; arched back; hunched posture.</td>
<td>Loss of weight and condition; sores on face; hair loss on tail.</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Eyes sunken and dull; changes in respiration; increased timidity; increased sleepiness; arched back; increased vocalization.</td>
<td>Weight loss; hair loss; scaly skin; dehydration; decreased timidity; unresponsive; excessive salivation (oral problems); increased barbering; loss of righting reflex; decreased vocalization; hypothermia</td>
</tr>
</tbody>
</table>
### Rabbit
Ocular discharge; protruding nictitans; photophobia; constipation or diarrhea; depression; facing back of cage; excessive self-grooming; stretched posture; early failure to eat and drink; dull attitude or increased aggression when handled; possible vocalization when handled; tooth grinding

###Tooth grinding; apparent sleepiness; dehydration; weight loss; fecal staining, wasting of lower back muscles; decreased production of night feces; unresponsive.

### Non-human primates
Generally very few signs, especially in the presence of humans; decreased activity; decreased food and water intake.

### Huddled or crouching posture, with hand folded over abdomen; clenching or grinding teeth; depression or increased restlessness; withdrawal from cage mates; increased (generally aggressive) attention from cage mates; anorexia; weight loss; decreased grooming.

### Dog
Decreased alertness; stiff posture; panting; biting, licking or scratching; increased aggression; increased vocalization; pacing.

### Unwillingness to move; crouching posture; depression or increased aggression; crying when handled or moved; increased restlessness.

### Cat
Increased aggression when approached; decreased food intake; licking.

### Hunched, crouching or stretched posture; increased aggression; anorexia; weight loss; vocalizing; wild escape behavior; unkempt appearance; pupillary dilation; stiff gait.

### Pig
Changes in gait or posture; increased efforts to avoid handling; increased squealing when approached or handled.

### Depression; unwillingness to move; attempts to hide; withdrawal from pen mates; anorexia.

### Sheep, Goat
Sheep are more stoic than goats; lying with legs extended; stamping feet; swaying stance; mild ataxia; restlessness or depression; depressed food intake; increased aggression on handling; guarding; tooth grinding.

### Rolling; frequently looking or kicking at abdomen; falling over; walking backward; rapid, shallow respiration; weight loss; tooth grinding; grunting; vocalization on handling (goats especially); rigidity; unwillingness to move.

### Bird
Increased escape behavior and vocalization when approached or handled.

### Eyelids partially closed; anorexia; ruffled, drooping, unkempt appearance; immobility when approached.

*Adapted from the University of Nebraska Medical Center “IACUC Guidelines for the Humane Care and Use of Live Vertebrate Animals,” 4th Edition, Appendix E: “Guidelines on the Recognition of Pain”.

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

<table>
<thead>
<tr>
<th>Type of pain</th>
<th>Severity</th>
<th>Examples of procedure</th>
<th>Duration analgesia is provided</th>
<th>Recommended analgesics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical</td>
<td>Mild</td>
<td>Punch biopsy, vascular cutdown</td>
<td>Once</td>
<td>Local +/- NSAID</td>
</tr>
<tr>
<td>Surgical</td>
<td>Moderate</td>
<td>Head cap, craniotomy, subcutaneous procedure</td>
<td>1 full day</td>
<td>Local with either NSAID or Narcotic</td>
</tr>
<tr>
<td>Surgical</td>
<td>Severe</td>
<td>Thoracotomy, laparotomy</td>
<td>3 full days</td>
<td>Local with both NSAID and Narcotic</td>
</tr>
<tr>
<td>Chronic</td>
<td>Mild-moderate</td>
<td>Arthritis</td>
<td>Long term</td>
<td>NSAID</td>
</tr>
</tbody>
</table>

41
Anesthetics and Analgesics used in Mice

The following table is provided as a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Mice</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane*)</td>
<td>To effect. In general, 3-4% induction, 1-3% maintenance; inhalation</td>
<td>Precision vaporizer, adequate ventilation or scavenging essential</td>
</tr>
<tr>
<td>Pentobarbital*</td>
<td>35 mg/kg IV 60 – 80 mg/kg IP</td>
<td>Cautions! Potentially significant cardiovascular and respiratory depression, variable response</td>
</tr>
<tr>
<td>Tribromoethanol (Avertin)</td>
<td>125-250 mg/kg IP</td>
<td>Store at 4°C; dark conditions</td>
</tr>
<tr>
<td>Ketamine* + Xylazine</td>
<td>80-100 mg/kg (K) 5-12mg/kg (X) IP</td>
<td>If animals appear to be responding to touch, or awakening, redose with 25-50% of the initial dose of ketamine.</td>
</tr>
<tr>
<td>Ketamine* + Xylazine + Acepromazine</td>
<td>80-100 mg/kg (K) 5-12mg/kg (X) + 1-3 mg/kg (A) IP</td>
<td></td>
</tr>
<tr>
<td>Hypothermia (Neonates &lt;4 days old undergoing minor surgical procedures only)</td>
<td>Place for 3 – 4 minutes in ice water or crushed ice</td>
<td>Pup placed in rubber sleeve, submerged to cervical area with resultant 10 minutes of anesthesia</td>
</tr>
</tbody>
</table>

| Analgesia in Mice                   |                                              |                                                                                           |
|-------------------------------------|                                              |                                                                                           |
| Buprenorphine* (Buprenex®)          | 0.05 – 0.1 mg/kg SC or IP                   | 4-6 hours of analgesia; do not use with tribromoethanol                                   |
| Buprenorphine SR* (ZooPharm)        | 1 mg/kg SC                                   | Up to 72 hours of analgesia                                                              |
| Carprofen                           | 2-5 mg/kg SC                                 | Up to 12 hours of analgesia                                                              |
| Ketoprofen                          | 5 mg/kg SC                                   | Up to 12 hours of analgesia                                                              |
| Meloxicam                           | 5-10 mg/kg SC                                | 12-24 hours respectively                                                                |
| Meloxicam SR                        | 4 mg/kg SC                                   | Up to 72 hours of analgesia                                                              |
| Lidocaine                           | 4 mg/kg SC or intralesional                  | Do not exceed 7mg/kg total                                                               |
| Bupivacaine                         | 2-2.5 mg/kg SC or intralesional             | Do not exceed 8 mg/kg total                                                               |

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

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Rats</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane*)</td>
<td>To effect. In general, 3-5% induction, 1-3% maintenance; inhalation</td>
<td>Precision vaporizer, adequate ventilation or scavenging essential</td>
</tr>
<tr>
<td>Pentobarbital*</td>
<td>30 – 40 mg/kg IV 40 – 60 mg/kg IP</td>
<td>Caution! Potentially significant cardiovascular and respiratory depression, variable response</td>
</tr>
<tr>
<td>Ketamine* + Xylazine + Acepromazine</td>
<td>20-50 mg/kg (K) + 2-10 mg/kg (X) + 0.5-1.5 mg/kg (A)</td>
<td></td>
</tr>
<tr>
<td>Ketamine* + Xylazine</td>
<td>40 – 100 mg/kg (K)+ 5 – 10 mg/kg (X) IP</td>
<td>30 – 45-minute duration; may supplement with ketamine only @ 25-50% dose. Reverse xylazine w/Yohimbine (SC or IP), 2mg/kg BW</td>
</tr>
<tr>
<td>Methohexital* (Brevital)</td>
<td>40 mg/kg IV or IP (1% solution)</td>
<td>15 - 20 minutes of anesthesia</td>
</tr>
</tbody>
</table>

Analgesia in Rats

| Analgesia in Rats | | |
|-------------------|----------------------|
| Buprenorphine SR* | 1 mg/kg SC | Up to 72 hours of analgesia |
| Buprenorphine* (Buprenex*) | 0.01 – 0.05 mg/kg SC or IP | From 6 – 8 hours of analgesia |
| Ketoprofen | 5.0 mg/kg SC | Up to 12 hours of analgesia |
| Carprofen | 5.0 mg/kg SC | Up to 12 hours of analgesia |
| Meloxicam | 1-2 mg/kg SC or PO | Once every 12-24 hours |

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 Hamsters

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Hamsters</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane®)</td>
<td>To effect. In general, 3-4% induction, 1-3% maintenance; inhalation</td>
<td>Precision vaporizer, adequate ventilation or scavenging essential</td>
</tr>
<tr>
<td>Ketamine*+Xylazine</td>
<td>80 – 100 mg/kg (K) + 7 – 10 mg/kg (X) IP</td>
<td></td>
</tr>
<tr>
<td>Ketamine*+ Dexmedetomidine</td>
<td>70 mg/kg (K) + 1 mg/kg (M) IP</td>
<td></td>
</tr>
</tbody>
</table>

Analgesia in Hamsters

<table>
<thead>
<tr>
<th>Analgesia in Hamsters</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine* (Buprenex®)</td>
<td>0.05 – 0.5 mg/kg SC</td>
<td>Between 6 – 12 hours of analgesia</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1-2 mg/kg SC</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Carprofen</td>
<td>5 mg/kg SC</td>
<td>Between 12-24 hours of analgesia</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>5 mg/kg SC</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Flunixin meglumine</td>
<td>2.5 mg/kg SC</td>
<td>Between 12-24 hours of analgesia</td>
</tr>
</tbody>
</table>

* - Controlled Substance

Anesthetics and Analgesics used in Guinea Pigs

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Guinea Pigs</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane®)</td>
<td>To effect. In general, 3-4% induction, 1-3% maintenance; inhalation</td>
<td>Precision vaporizer required, scavenging required</td>
</tr>
<tr>
<td>Ketamine* + Xylazine</td>
<td>40-50 mg/kg (K) + 5 mg/kg (X) IP</td>
<td>Up to 60 minutes of anesthesia</td>
</tr>
</tbody>
</table>

Analgesia in Guinea Pigs

<table>
<thead>
<tr>
<th>Analgesia in Guinea Pigs</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carprofen (Rimadyl®)</td>
<td>4 mg/kg SC</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>1 mg/kg SC, IM</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Buprenorphine* (Buprenex®)</td>
<td>0.05 mg/kg SC</td>
<td>6-12 hours of analgesia</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.1-0.3 mg/kg SC</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Flunixin meglumine</td>
<td>2.5 mg/kg SC</td>
<td>Between 12-24 hours of analgesia</td>
</tr>
</tbody>
</table>

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

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Rabbits</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane*)</td>
<td>To effect. In general, 3-5% induction, 1-3% maintenance; inhalation</td>
<td>Precision vaporizer, adequate ventilation or scavenging essential</td>
</tr>
<tr>
<td>Ketamine* + Xylazine</td>
<td>35 – 50 mg/kg (K) + 5 – 10 mg/kg (X) IM</td>
<td>Minor procedures; up to 45 minutes of anesthesia; can supplement with ketamine @ 25-50% dose</td>
</tr>
<tr>
<td>Ketamine* + Dexmedetomidine</td>
<td>25 mg/kg (K) + 0.25 mg/kg (D) IM</td>
<td></td>
</tr>
<tr>
<td>Ketamine* + Acepromazine</td>
<td>25-50 mg/kg (K) + 0.25-1 mg/kg (A) IM</td>
<td></td>
</tr>
</tbody>
</table>

## Analgesia in Rabbits

<table>
<thead>
<tr>
<th>Analgesia in Rabbits</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butorphanol* (Torbutrol* 0.5mg/ml)</td>
<td>0.1 – 0.5 mg/kg SC, IM or IV</td>
<td>Up to 4 hours of analgesia</td>
</tr>
<tr>
<td>Buprenorphine* (Buprenex*)</td>
<td>0.01-0.05 mg/kg SC</td>
<td>Between 6 – 12 hours of analgesia</td>
</tr>
<tr>
<td>Buprenorphine SR*</td>
<td>0.12 mg/kg SC</td>
<td>Between 48-72 hours of analgesia</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2-4 mg/kg SC or PO</td>
<td>Between 12 hours of analgesia</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>2 mg/kg SC initially, followed with 0.2-0.5 mg/kg daily</td>
<td>Up to 24 hours of analgesia, treatment should not exceed 5 days</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.5 mg/kg SC</td>
<td>Once every 12 hours</td>
</tr>
<tr>
<td>Banamine</td>
<td>1.1 mg/kg IM or SC</td>
<td>Up to 12 hours of analgesia</td>
</tr>
<tr>
<td>Fentanyl patch *</td>
<td>Under 4 kg: use 12 mcg/hr =&gt; 4 kg: Use 20 mcg/hr</td>
<td></td>
</tr>
</tbody>
</table>

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

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Dogs</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane*)</td>
<td>To effect. In general, 3-5% induction, 1-4% maintenance; inhalation</td>
<td>Precision vaporizer, adequate ventilation or scavenging essential</td>
</tr>
<tr>
<td>Ketamine* + Diazepam</td>
<td>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)</td>
<td>Premedicate with an anticholinergic Anesthesia can be maintained with inhalant anesthetic (e.g., isoflurane)</td>
</tr>
<tr>
<td>Ketamine* + Midazolam</td>
<td>5-10 mg/kg (K) + 0.1-0.5 mg/kg (M) IV</td>
<td>Minor procedures; premedicate with anticholinergic</td>
</tr>
<tr>
<td>Telazol** (Tiletamine + Zolazepam)</td>
<td>6 – 13 mg/kg IM</td>
<td>Up to 1 hour of anesthesia</td>
</tr>
<tr>
<td>Propofol*</td>
<td>4 mg/kg IV to effect (give slowly)</td>
<td></td>
</tr>
</tbody>
</table>

Analgesia in Dogs

| 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 |
| Deracoxib | 1-2 mg/kg PO | 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; place 12 hours prior to anticipated pain; do not apply heat to patch (e.g., from heating pads). |

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* | 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 |
**Anesthetics and Analgesics used in Cats**

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Cats</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane®)</td>
<td>To effect. In general, 3-5% induction, 1-3% maintenance; inhalation</td>
<td>Precision vaporizer, adequate ventilation or scavenging essential</td>
</tr>
<tr>
<td>Ketamine* + Diazepam</td>
<td>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)</td>
<td>Premedicate with an anticholinergic&lt;br&gt;Anesthesia can be maintained with inhalant anesthetic (e.g., isoflurane)</td>
</tr>
<tr>
<td>Dexmedetomidine, Ketamine, Butorphanol</td>
<td>0.0325 mg/kg IM (D) + 6.5 mg/kg IM (K) + 0.65 mg/kg IM (B)</td>
<td>Takes effect in 5-10 minutes, can administer another ½ dose if necessary. Reverse with 1/3 total volume with atipamezole</td>
</tr>
<tr>
<td>Propofol*</td>
<td>4 mg/kg IV to effect (give slowly)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analgesia in Cats</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine*</td>
<td>0.05-0.4 mg/kg IM or SC</td>
<td>Up to 4 hours analgesia; caution, mania and excitation with overdose</td>
</tr>
<tr>
<td>Robenacoxib (Onsior®)</td>
<td>1 mg/kg PO</td>
<td>Once every 24 hours for maximum of three days</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.3 mg/kg PO or SC</td>
<td>Give once only</td>
</tr>
<tr>
<td>Buprenorphine* (Buprenex®)</td>
<td>0.005 – 0.01 mg/kg SC or IM</td>
<td>Up to 12 hours analgesia</td>
</tr>
<tr>
<td>Fentanyl patch*</td>
<td>&lt;2.5 kg body weight = ½ of 25 μg/hr patch; &gt;2.5 kg bdy wt = 25 μg/hr patch</td>
<td>Each up to 5 days analgesia; place 8 hours prior to anticipated pain; do not apply heat to patch (e.g., from heating pads)</td>
</tr>
</tbody>
</table>

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 USF 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 Pigs**

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Analgesia in Pigs</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen (Tylenol)</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>

* - Controlled Substance
### Anesthesia in Pigs

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

### Analgesia in Pigs

<table>
<thead>
<tr>
<th>Analgesia</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>10 mg/kg PO</td>
<td>Up to 6 hours of analgesia; use enteric-coated tablet</td>
</tr>
<tr>
<td>Flunixin Meglumine (Banamine®)</td>
<td>1-4 mg/kg SC or IM</td>
<td>Up to 12 hours of analgesia</td>
</tr>
<tr>
<td>Buprenorphine* (Buprenex®)</td>
<td>0.01 - 0.02 mg/kg IM</td>
<td>Up to 12 hours of analgesia</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>1.0 – 3.0 mg/kg SC</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2-3 mg/kg SC, PO, IM q 12 hours</td>
<td>Up to 12 hours of analgesia</td>
</tr>
<tr>
<td>Buprenorphine SR*</td>
<td>0.12-0.2 mg/kg SC</td>
<td>Up to 72 hours of analgesia</td>
</tr>
<tr>
<td>Hydromorphone*</td>
<td>0.1-0.2 mg/kg IV, IM, or SC q 2-4 hours</td>
<td>Up to 4 hours of analgesia</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.1-0.4 mg/kg PO, SC, or IM q 12-24 hours</td>
<td>Between 12-24 hours of analgesia</td>
</tr>
<tr>
<td>Fentanyl* Patches</td>
<td>&lt;30 kg use 25 mcg patch; 30-50 kg use 50 mcg patch; &gt;50 kg use 75 mcg patch</td>
<td>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.</td>
</tr>
</tbody>
</table>

---

**Note:** Malignant hyperthermia (MH) is commonly reported in swine. The first cardinal clinical sign of MH is an elevation in end-tidal CO₂. 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 (particularly with halothane, isoflurane, or ethrane), 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

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Sheep &amp; Goats</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane®)</td>
<td>To effect. In general, 3-5% induction, 1-4% maintenance; inhalation</td>
<td>Precision vaporizer, adequate ventilation or scavenging essential</td>
</tr>
<tr>
<td>Diazepam + Ketamine*</td>
<td>0.2 mg/kg (D) + 3 mg/kg (K) IV</td>
<td>Up to 20 minutes of anesthesia; for minor procedures</td>
</tr>
<tr>
<td>Xylazine + Ketamine*</td>
<td>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)</td>
<td>Up to 20 minutes of anesthesia; for minor procedures Up to 45 minutes of anesthesia</td>
</tr>
<tr>
<td>Telazol**</td>
<td>2-4 mg/kg IV</td>
<td>Up to 30 minutes of anesthesia</td>
</tr>
<tr>
<td>Xylazine + Telazol**</td>
<td>0.1 mg/kg (X) + 4 mg/kg (T) IM 0.05 mg/kg (X) + 1 mg/kg (T) IV</td>
<td>Up to 60 minutes of anesthesia</td>
</tr>
<tr>
<td>Propofol*</td>
<td>2-6 mg/kg IV to effect (give slowly)</td>
<td>Can add 2-6 mg/kg ketamine* IV if propofol alone is inadequate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analgesia in Sheep &amp; Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Flunixin Meglumine (Banamine)</td>
</tr>
<tr>
<td>Buprenorphine* (Buprenex*)</td>
</tr>
<tr>
<td>Buprenorphine SR</td>
</tr>
<tr>
<td>Fentanyl* Patches</td>
</tr>
<tr>
<td>Bupivicaine</td>
</tr>
</tbody>
</table>

### Sedation in Sheep & Goats

<table>
<thead>
<tr>
<th>Sedation in Sheep &amp; Goats</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acepromazine</td>
<td>0.05-0.1 mg/kg IM, SC</td>
<td>Moderate sedation</td>
</tr>
<tr>
<td>Butorphanol + Diazepam*</td>
<td>0.05-0.1mg/kg (B) + 0.05-0.2 mg/kg (D) IV</td>
<td>Up to 4 hours of analgesia</td>
</tr>
<tr>
<td>Diazepam*</td>
<td>0.2-1 mg/kg IV, IM</td>
<td>Light sedation</td>
</tr>
<tr>
<td>Ketamine*</td>
<td>20 mg/kg IM</td>
<td>Moderate to heavy sedation</td>
</tr>
<tr>
<td>Dexmedetomidine*</td>
<td>0.015 mg/kg IM</td>
<td>Moderate sedation</td>
</tr>
<tr>
<td>Midazolam* + Ketamine*</td>
<td>0.5 mg/kg (M) + 4 mg/kg (K)</td>
<td>Heavy sedation</td>
</tr>
<tr>
<td>Midazolam*</td>
<td>0.3-0.5 mg/kg IM or IV</td>
<td>Light sedation</td>
</tr>
</tbody>
</table>
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

The following table is a reference only, for use by Rutgers University faculty and staff.

<table>
<thead>
<tr>
<th>Anesthesia in Macaca spp</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane*)</td>
<td>To effect. In general, 3-5% induction, 1-3% maintenance; inhalation</td>
<td>Precision vaporizer, adequate scavenging essential</td>
</tr>
<tr>
<td>Ketamine + Diazepam*</td>
<td>15 mg/kg (K) + 1.0 mg/kg (D) IM</td>
<td>30-40 minutes of anesthesia</td>
</tr>
<tr>
<td>Ketamine* + Xylazine</td>
<td>10 mg/kg (K) + 0.25-2.0 mg/kg (X) IM</td>
<td>30-140 minutes of anesthesia; duration is a function of the xylazine dose</td>
</tr>
<tr>
<td>Ketamine* + Dexametomidine</td>
<td>4-5 mg/kg (K) + 0.015-0.05 mg/kg (D) IM</td>
<td>Up to 60 minutes of anesthesia</td>
</tr>
<tr>
<td>Pentobarbital*</td>
<td>20-30 mg/kg IV</td>
<td>30-60 minutes of anesthesia: reduce dose by 1/3 to 1/2 after administration of ketamine</td>
</tr>
<tr>
<td>Thiopental*</td>
<td>15-20 mg/kg IV</td>
<td>5-10 minutes of anesthesia</td>
</tr>
<tr>
<td></td>
<td>5-7 mg/kg IV (induction)</td>
<td>After administration of ketamine</td>
</tr>
<tr>
<td>Telazol**</td>
<td>4-6 mg/kg IM</td>
<td>45-60 minutes of anesthesia</td>
</tr>
</tbody>
</table>

Analgesia in Macaca spp

<table>
<thead>
<tr>
<th>Analgesia in Macaca spp</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>10 mg/kg PO</td>
<td>Up to 6 hours of analgesia</td>
</tr>
<tr>
<td>Aspirin</td>
<td>20mg/kg PO</td>
<td>6 to 8 hours</td>
</tr>
<tr>
<td></td>
<td>125 mg/kg rectal suppository</td>
<td>&lt; 24 hours</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>2 mg/kg IM SID to BID</td>
<td>Up to 12 hours of analgesia</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2-4 mg/kg PO, SC</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Ketorolac</td>
<td>15-30 mg/kg IM</td>
<td></td>
</tr>
<tr>
<td>Flunixin Meglumine</td>
<td>2-4 mg/kg, SC</td>
<td></td>
</tr>
<tr>
<td>(Banamine)</td>
<td></td>
<td>Up to 24 hours of analgesia; only administer postoperatively to conscious animals</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.2 mg/kg SQ or PO loading dose, then 0.1 mg/kg thereafter</td>
<td>Up to 24 hours of analgesia</td>
</tr>
<tr>
<td>Naproxen</td>
<td>10 mg/kg PO</td>
<td>Up to 12 hours of analgesia</td>
</tr>
<tr>
<td>Oxymorphine*</td>
<td>0.15 mg/kg SC, IM, IV</td>
<td>Up to 6 hours of analgesia</td>
</tr>
<tr>
<td>Meperidine*</td>
<td>2-4 mg/kg IM</td>
<td>Up to 4 hours of analgesia</td>
</tr>
<tr>
<td>Morphine*</td>
<td>1-2 mg/kg SC, IM</td>
<td>Up to 4 hours of analgesia</td>
</tr>
<tr>
<td>Buprenorphine* (Buprenex*)</td>
<td>0.01-0.03 mg/kg IM</td>
<td>Up to 8 hours of analgesia</td>
</tr>
</tbody>
</table>

Sedation in Macaca spp

<table>
<thead>
<tr>
<th>Sedation in Macaca spp</th>
<th>Dose &amp; Route</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acepromazine</td>
<td>0.2 mg/kg IM</td>
<td>Moderate sedation</td>
</tr>
<tr>
<td>Diazepam*</td>
<td>1.0 mg/kg IM</td>
<td>Light to moderate sedation</td>
</tr>
<tr>
<td>Ketamine*</td>
<td>5-20 mg/kg IM</td>
<td>Moderate sedation, immobilization</td>
</tr>
<tr>
<td>Xylazine</td>
<td>0.25-0.5 mg/kg IM</td>
<td>Light to moderate sedation</td>
</tr>
</tbody>
</table>
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

**VI. References**

Boogaerts J; Declercq A; Lafont N; Benameur H; Akodad EM; Dupont JC; Legros FJ. Toxicity of bupivacaine encapsulated into liposomes and injected intravenously: comparison with plain solutions. Anesthesia and Analgesia, 1993 Mar, 76(3):553-5.


Wong, P.; Young, S. COMPMED archives, 1999.
I. **Purpose:** This document covers the procedures to be followed by all Rutgers University investigators and staff 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 Principal Investigator and associated study personnel 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 Institutional Animal Care and Use Committee (IACUC) reviews all proposed and current animal use procedures to ensure that Rutgers University meets the legal and ethical obligations with regard to animal research.

The goal of the investigator 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. In planning, implementation, and continuous review of animal studies, consideration of the three "Rs" of animal research should always be applied to each pain or distress event. Can the event that may cause pain or distress, be replaced, refined to lessen pain or distress or the number of animals involved reduced? If not, humane endpoints should be established for expected and potential events. The protocol author should consider various sources to assist in developing a study and writing the protocol. For example, familiarity with the pharmacological side effects of drugs (classes) prior to the experiments can be extremely helpful in this endeavor. 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, including Principal Investigators or designees, veterinarians, and animal care staff are responsible for implementation and oversight of these procedures.

IV. **Definitions**

a. The **experimental endpoint** of a study is when the scientific aims and objectives have been reached.
b. 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. *(Guide 2011, p27)*

V. Methods

**Summary of selected criteria for euthanasia.**

If any animal shows one or more of these clinical signs, they must be euthanized unless pre-approved by the IACUC.

- Weight loss ≥10% within one week (considered rapid weight loss)
- Weight loss ≥ 20% over any time period
- Body condition scoring (BCS) ≤1.5
- 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
- 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

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 personnel 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

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

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 with the PI to understand scientific objectives and limitations, and help in developing a stronger scientific and humane protocol

Studies that achieve their scientific objective prior to their anticipated termination time should be terminated earlier. The relevant humane endpoints that are identified should be described when the protocol is being planned and incorporated into the protocol. The appropriate personnel should be informed and trained in their roles in the study. If an unexpected event occurs during a study, the PI and Veterinary Staff need to address the situation and then review the event. If warranted, they should then submit an amendment to enhance the endpoint section of the protocol.

Parameters to consider when formulating humane endpoints include:

a. Changes in external physical appearance or other clinical signs

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

Animals should be examined regularly by appropriately trained staff. 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.

Endpoints in tumor studies include tumor size greater than 10% of body weight, mean tumor diameter exceeding 20mm in mice, 40mm in rats, ulceration of the tumor, any interference with normal body functions and movement. Refer to the Cancer Studies / Tumor Models document for more details.

b. Physiological changes

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

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 is usually secondary to a change in food and water consumption, which should also be closely monitored by animal care staff. It may be due to metabolism changes for sub-acute or chronic studies. 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 and approved by the IACUC.

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 ≤ 1.5

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** (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.

c. Diagnostic laboratory testing

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

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.”

d. 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)

Moribund
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 should always be indicated if it does occur.

Death as an endpoint
There are several types of studies where death of the animal may historically have been the experimental endpoint. This requirement is now questioned, in light of recent studies that have shown efficacy of biomarkers that can be used in place of death as an endpoint. These areas include regulatory toxicology, diagnostic toxicology, acute toxicity studies in research, infectious disease studies, microorganism virulence challenge studies, vaccine efficacy trials, cancer research, and cancer treatment evaluation. 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.
e. Guidelines for Some of the Potential Signs Associated with Pain or Distress in Rodents and Rabbits

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

A variety of 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 aged 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.

VI. General Endpoint References


d. Institute for Laboratory Animal Research Journal (2000), Humane Endpoints for Animals Used in Biomedical Research and Testing. 41: No. 2


g. OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation (2000)

http://www.olis.oecd.org/olis/2000doc.nsf/4f7adc214b91a685c12569fa005d0ee7/c125692700623b74c12569bb005aa3d5/$FILE/00087372.pdf


I. Purpose: This document was designed to provide investigators with reference values related to the administration and collection of fluids (including 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 based on personal experience of the Veterinary Staff. This document contains three sections: (1) recommendations regarding dosing of compounds based on common routes of administration. Included are typical injection volumes and maximum allowable 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 (defined as any needle greater than 16ga).

III. Responsibilities: This document applies to anyone 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 = epidural (outside of meninges)
IP = intraperitoneal  ICV = intracerebroventricular (into lateral ventricle of brain)
RO = retro-orbital

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 close to the typical volume (value on left side under body weight on chart). Note that compounds cannot be delivered in a volume greater than the maximum value listed on the right column under body weight (highlighted in red) without prior Institutional Animal Care and Use Committee (IACUC) approval. Please see the charts below.

Mice (values across top are body weights in grams)

All values listed on chart are in MICRO liters (µl); value on left is typical volume, volume on right (highlight in red) is the maximum volume allowed by that route. * = requires IACUC approval

<table>
<thead>
<tr>
<th>route</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>≥35</th>
<th>needle</th>
</tr>
</thead>
<tbody>
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<td>PO</td>
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<td>150</td>
<td>750</td>
<td>200</td>
<td>1000</td>
<td>250</td>
</tr>
<tr>
<td>IN</td>
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<td>50</td>
<td>35</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>SC</td>
<td>70</td>
<td>400</td>
<td>105</td>
<td>600</td>
<td>140</td>
<td>800</td>
<td>175</td>
</tr>
<tr>
<td>RO</td>
<td>20</td>
<td>60</td>
<td>30</td>
<td>90</td>
<td>40</td>
<td>120</td>
<td>50</td>
</tr>
<tr>
<td>ID</td>
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<td>100</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>IP</td>
<td>150</td>
<td>800</td>
<td>225</td>
<td>1200</td>
<td>300</td>
<td>1600</td>
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<td>1.5</td>
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</tr>
<tr>
<td>IT</td>
<td>5</td>
<td>20</td>
<td>10</td>
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<td>40</td>
<td>10</td>
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<td>10</td>
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<td>50</td>
<td>75</td>
<td>100</td>
<td>125</td>
<td>150</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>IV (slow)</td>
<td>250</td>
<td>375</td>
<td>500</td>
<td>625</td>
<td>750</td>
<td>875</td>
<td>875</td>
</tr>
</tbody>
</table>

Rats (values across top are body weights in grams)

All values listed on chart are in MILLI liters (ml); value on left is typical volume, volume on right (highlighted in red) is the maximum volume allowed by that route.

<table>
<thead>
<tr>
<th>route</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>350</th>
<th>400</th>
<th>450</th>
<th>500</th>
<th>needle</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>1</td>
<td>4</td>
<td>1.5</td>
<td>6</td>
<td>2</td>
<td>8</td>
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<td>10</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>IN</td>
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<td>0.35</td>
<td>0.50</td>
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<td>0.50</td>
<td>0.35</td>
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<tr>
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<td>3</td>
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</tr>
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<td>0.05</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>IP</td>
<td>1</td>
<td>2</td>
<td>1.5</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2.5</td>
<td>5</td>
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<td>6</td>
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<td>0.03</td>
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<td>0.025</td>
<td>0.05</td>
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</tr>
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<td>0.03</td>
<td>0.04</td>
<td>0.038</td>
<td>0.05</td>
<td>0.05</td>
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</tr>
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<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
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</tr>
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<td>0.018</td>
<td>0.02</td>
</tr>
<tr>
<td>IV (bolus)</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
</tr>
<tr>
<td>IV (bolus)</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>1.25</td>
<td>1.5</td>
<td>1.75</td>
<td>2</td>
<td>2.25</td>
<td>2.5</td>
<td>≥23ga</td>
</tr>
<tr>
<td>IV (slow)</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>≥23ga</td>
</tr>
</tbody>
</table>
Notes on specific routes:

**Oral gavage (PO)** is performed using a feeding needle only (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 or between the shoulder blades.

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

**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.

**IM injections** are limited to the quadriceps femoris or biceps femoris muscle groups. Due to the small size of mice, IM injections are not permitted in mice without prior IACUC approval, due to potential for nerve damage and muscle necrosis/irritation. No more than two IM injections are permitted every 24hrs for other rodents.

**IT, ICV injections** should be given over at least 1-2min per 10ul in mice and no greater than 0.25ml per minute in rats.

**Intratracheal injections** must be administered at doses lower than or within the range of 50-100 µl in mice, 100-300 µl in rats. All intratracheal injections must be performed under heavy sedation or general anesthesia.

A **bolus IV 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 IV injection** is delivered over a 5-10 minute period.

For **IP 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 2-4wks. No more than 20% CBV can be removed at one time and requires sufficient recovery time (see below). Animals MUST have appropriate recovery time after collection, 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.)
- frequency of sampling
- quantity of blood required
- health status of the animal(s)
- quality of sample (sterility, tissue contamination, etc.)
- training/experience of collector

Blood sample volume ranges based on body weight:

<table>
<thead>
<tr>
<th>BW(g)</th>
<th>CBV (ml)</th>
<th>1% (ml)</th>
<th>10% (ml)</th>
<th>20% (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.10-1.40</td>
<td>0.011-0.014</td>
<td>0.11-0.14</td>
<td>0.22-0.28</td>
</tr>
<tr>
<td>25</td>
<td>1.37-1.75</td>
<td>0.014-0.018</td>
<td>0.14-0.18</td>
<td>0.28-0.36</td>
</tr>
<tr>
<td>30</td>
<td>1.65-2.10</td>
<td>0.017-0.021</td>
<td>0.17-0.21</td>
<td>0.34-0.42</td>
</tr>
<tr>
<td>35</td>
<td>1.93-2.45</td>
<td>0.019-0.025</td>
<td>0.19-0.25</td>
<td>0.38-0.50</td>
</tr>
<tr>
<td>40</td>
<td>2.20-2.80</td>
<td>0.022-0.028</td>
<td>0.22-0.28</td>
<td>0.44-0.56</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>6.88-8.75</td>
<td>0.069-0.088</td>
<td>0.69-0.88</td>
<td>1.38-1.76</td>
</tr>
<tr>
<td>150</td>
<td>8.25-10.50</td>
<td>0.082-0.105</td>
<td>0.82-1.0</td>
<td>1.64-2.0</td>
</tr>
<tr>
<td>200</td>
<td>11.00-14.00</td>
<td>0.11-0.14</td>
<td>1.1-1.4</td>
<td>2.2-2.8</td>
</tr>
<tr>
<td>250</td>
<td>13.75-17.50</td>
<td>0.14-0.18</td>
<td>1.4-1.8</td>
<td>2.8-3.6</td>
</tr>
<tr>
<td>300</td>
<td>16.50-21.00</td>
<td>0.17-0.21</td>
<td>1.7-2.1</td>
<td>3.4-4.2</td>
</tr>
<tr>
<td>350</td>
<td>19.25-24.50</td>
<td>0.19-0.25</td>
<td>1.9-2.5</td>
<td>3.6-5.0</td>
</tr>
</tbody>
</table>

Blood collection recovery times:

<table>
<thead>
<tr>
<th>% CBV removed</th>
<th>recovery period</th>
<th>% CBV removed in 24hrs</th>
<th>recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>24hrs</td>
<td>1%</td>
<td>24hrs</td>
</tr>
<tr>
<td>10%</td>
<td>2 weeks</td>
<td>10%</td>
<td>2 weeks</td>
</tr>
<tr>
<td>20%</td>
<td>4 weeks</td>
<td>20%</td>
<td>3 weeks</td>
</tr>
</tbody>
</table>

Blood collection sites in rodents:

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

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.

**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 (≤16ga), 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 or 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 analgesia for at least 12hrs postoperatively.

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 the Veterinary Staff.
VI. References


I. **Purpose:** The purpose of this document is to provide investigators with guidelines in regard to 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 of food under general anesthesia (usually <12hrs).

II. **Introduction:** Language in 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 require food or fluid restriction/regulation include:
- Behavioral research protocols may require withholding food and/or water in order to train animals to perform a task, while providing food or water as a reward for the correct behavior
- Nutrition studies may require altering the levels of specific nutrients in the daily diet
- Food restriction is common for some sedentary laboratory species in order to control obesity or maximize life-span (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 (Fox 2002). 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

fasting – the removal of food (but typically not water) prior to an experimental manipulation such as surgery or imaging; 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

Food and water restriction/regulation may be used in studies for a short period of time, e.g. as a preparation for anesthesia or a surgical procedure, or as a prelude to performing a pharmacokinetic study. Long term food or water restriction/regulation is used mostly as a motivator for behavioral studies. The following items are the minimum requirements for all protocols that use food or fluid restriction/regulation:

i. 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.

ii. The maximum period of restriction/regulation must be clearly stated in the protocol. Depending on the species, an acceptable fasting period in preparation for surgery is usually no longer than 12 hours for larger animals, no longer than 4 hours in rodents.

iii. 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. Weight logs may be kept in the animal room or if the information is easily retrievable it may be kept as part of the regular research records.

iv. 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 state of hydration on a log sheet and have it available for review.

v. 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 and have them available in the laboratory for review.

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.
i. Baseline body weight of animal(s) must be measured before food or water restriction/regulation is begun.

ii. For juvenile rodents (<21d), body weight is compared to a comparable animal’s body weight (accounting for sex, strain, etc.).

iii. Restriction must be based on a measurable parameter such as percentage of ad lib intake or duration of restriction.

iv. In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction/regulation, is recommended.

v. 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.

vi. 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.

vii. For animals on lifetime food restriction (such as maintaining 85% body weight), animals must be weighed 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.

viii. Water restricted/regulated animals must be monitored at least daily, including weekend and holidays; skin turgor must be used to assess hydration status.

ix. 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.

1. For food restriction/regulation: a rodent may not lose more than 20% of body weight of age-strain-sex matched animal.
   a. 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.
   b. 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.

2. for fluid restriction/regulation:
   a. Rodents on fluid restriction/regulation with a weight loss of 10% of baseline weight are considered clinically dehydrated and should be allowed to freely drink water without interruption. 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.

x. Individual cages must be marked with a “food / water restriction/regulation” card (see Appendix I); groups of cages (>5) can be labeled with food or fluid restriction/regulation sticker. Contact the Facility Supervisor for details.

xi. A daily log sheet must be maintained for each box of animals on restricted/regulated food or water protocols and kept with the animals in the animal holding room.

1. The log sheet must include: current protocol number, PI name, contact name, email or phone number, type of restriction, length of restriction, start / stop dates, number of cages / animals,
initial body weight(s), weekly body weight measurements, and animal health conditions (appearance, behavior). A recommended log sheet is included at the end of this document.

xii. Research staff is responsible for monitoring animals on food or fluid restriction/regulation studies.

xiii. Research staff must be trained and competent to evaluate the animal’s condition.

VI. References


Appendix I – Cage Card for Food / Fluid Restriction

<table>
<thead>
<tr>
<th>Date</th>
<th>Observation</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Rutgers Rodent Food/Fluid Restriction/Regulation Record Form

<table>
<thead>
<tr>
<th>Protocol number:</th>
<th>Species: Mice  Rats  Other:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Investigator:</td>
<td>Restriction: Food  Water  Both</td>
</tr>
<tr>
<td>Laboratory member:</td>
<td>Duration of restriction/regulation:</td>
</tr>
<tr>
<td>Laboratory phone:</td>
<td>Reason for restriction/regulation: __________________________</td>
</tr>
<tr>
<td>After hours phone:</td>
<td></td>
</tr>
<tr>
<td>Has this restriction been approved by the IACUC? Y / N If 'no' or unsure contact IACUC before proceeding</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Animal ID</th>
<th>Body wt (g)</th>
<th>Time food or water removed</th>
<th>Time food/water offered</th>
<th>Skin turgor [1]</th>
<th>Comments</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

[1] Skin turgor: A measure of the skin's elasticity, used to assess fluid status.

70
I. **Purpose:** This policy applies to all surgical procedures performed at Rutgers University on mammalian 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

Surgery has great potential for causing pain, distress, tissue damage, and post-operative infection in animals if not performed properly. Therefore, stringent guidelines for training, surgical facilities, asepsis, surgical preparation, anesthesia, intra-operative records, analgesia, surgical technique, and post-operative monitoring have been established. 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**

   a. **Surgery** - any procedure that exposes tissues normally covered by skin or mucosa

   b. **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)

   c. **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)

   d. **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.
e. **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

f. **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.”

“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 interspecies 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. Generally, this will require the veterinarian 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 surgery 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 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.ii) 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.iii for more information). Instrument cleaning and pack preparation may also occur in this area but cannot occur in the operating room.
c. Surgical Preparation

i. 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.

1. 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.

Cold chemical sterilants may be used effectively for many items. 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.

Note: Rutgers does not consider alcohol to be a sterilizing agent, as it is only an effective sterilant under prolonged exposure times.

ii. 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, however. 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.

Animal Preparation - Farm Animal Species

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.
- Cover the hooves of large animals before the animal is brought into surgery. This can be done with exam gloves, small bags, paper, etc.
- Anesthetize animals in the animal prep area, and carry into surgery or move on a cart. Do not allow animals to walk on the surgery room floor.

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.

A common mistake is to make the drape too small. The larger the sterile field you create, the easier it will be to avoid breaks in sterile technique. Novice surgeons should err on the side of a too-large drape.

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

iii. **Surgeon**

Shoe covers are required for all persons in the surgery suite, at all times. Change shoe covers if worn outside the surgery suite, before re-entering.

Special surgical attire (scrubs) is recommended.

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 the recommendation for surgical scrubs. 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.

Other personnel in the surgery room during surgery must wear a cap, mask, lab coat, and shoe covers.

### Minimal Surgeon Preparation:
- Changing into surgical scrubs from street clothes
- Thorough hand scrub with a surgical scrub brush and antiseptic soap
- Sterile gown
- Sterile gloves
- Cap and mask
- Shoe covers

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 time, trauma, and trash.

- **Time** 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.
- **Trash** refers to contamination by 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 patient; 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 disinfectants such as alcohol, iodophors, quaternary ammonium, and phenolic compounds are not acceptable for use in surgical procedures.

e. **Anesthesia** – Anesthesia must be adequate to maintain the animal in a surgical plane of anesthesia for the duration of the procedure. 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 regard appropriate choice of anesthetic(s). For further information on anesthetics, refer to the IACUC document “Anesthesia and Analgesia”. 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 after each use. Canisters should be discarded in accordance to manufacturer’s recommendations, and should be discarded in the regular trash or as directed by REHS. CO2 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 15 min if using gas anesthesia, as needed for injectables)
- Vital signs such as heart rate, respiratory rate, body temperature, oxygen saturation, end tidal CO₂, blood pressure (updated every 15 min)
  - 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 animals. 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, all records are collected and stored with the CMR veterinary staff (research staff may retain copies for their records). Records for an individual protocol are maintained for 3 years from the date of termination of the protocol.

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 document 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.”


Immediate/acute post-operotive period:
Endotracheal tubes should be kept in place as long as possible; they must be removed when 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 may not be left alone nor unobserved.

The animal must be monitored minutes 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 warming devices as well as turning the patient 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 are to be closely observed for the initial 24-72 hour post-surgical procedure period. It is important to assess whether or not the animal has returned to normal behavior. Animals which do not return to normal often have surgical-related infections/complications and require re-evaluation. Animals should 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 <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 animals 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 drugs (including injected products like saline) may be used for non-survival surgery.**

VII. **References**


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 (eg. xenograft or allograft models)
2) Spontaneous, naturally occurring tumors (eg. geriatric tumors or thymoma in NOD-SCID mice)
3) Induced tumors in mutant mice (eg. Cre-lox mice, tamoxifen induced models)

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. **Definitions:** none

V. **Methods**

a. **Pathogen testing of tumor cell lines** - All rodent cell lines or human cells/tumors that have been passaged through rodents must be tested for infectious agents before use in animals at Rutgers.

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.
- Use of inhalation anesthetic prior to cell injections is recommended for safety of personnel.
• Tumor injection site(s) must be disinfected using 70% alcohol before injection.
• A maximum of two (2) tumor injections permitted for each animal.

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

i. Tumor size
  1. Mice: Single tumors exceeding 2cm in any dimension, or multiple tumors with a combined dimension of ≥2cm
  2. Rats: Single tumors exceeding 4cm in any dimension, or multiple tumors with a combined dimension of ≥4cm
  3. 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.

ii. Tumor volume
  1. Spherical tumors: \(\frac{\text{width}^2 \times \text{length}}{2}\)
  2. Ellipsoid tumors: \(\frac{\pi}{6} \times \text{length} \times \text{width} \times \text{height}\)
     Animals with tumor volumes that exceed 1700 mm\(^3\) in mice or 3400 mm\(^3\) in rats must be euthanized (volume of all tumors on a single animal).

iii. Ulcerated/necrotic tumors – animal must be euthanized regardless of tumor volume or weight.

iv. 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 calculate the tumor weight (calculated tumor weight):

\[\frac{d^2 \times (D/2)}{1000} = \frac{1}{2}(d^2 \times D) / 1000\text{ (alternative formula) = calculated tumor weight (gm)}\]

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

\[1000\text{mg} = 1000\text{mm}^3 = 1\text{cm}^3 = 1\text{cc} = 1\text{gm}\]

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

\[(\text{calculated tumor weight (gm)} / \text{adjusted or calculated body weight (gm)}) \times 100 = \text{tumor weight expressed as percentage of body weight}\]

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

Example:

Measurement calculation of tumor weight in a rat using a caliper:

d (width) = 28.3 mm
D (length) = 50 mm

tumor weight = \((28.3\text{mm})^2 \times (50\text{mm}/2) = 800.89 \times 25 = 20022.25\text{mm}^3 / 1000 = 20\text{cm}^3 = 20\text{gm}\]
If the rat weighs 220 g with the tumor and the calculated tumor weight is 20gm, the adjusted rat weight is 200gm (weight of rat without the tumor).

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

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

**v. Body Condition Score (BCS)** – Rodents with a BCS ≤ 1.5 must be euthanized; refer to the Humane Endpoints document for details regarding body condition scoring.

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

**vii. Humane Endpoints IACUC document** - 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 (≥16ga), 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 tumor size, 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. tumor diameter ≥1.0cm in mice or BCS ≤2) must be monitored daily, including holidays and weekends.

- Tumor measurement: tumor size must be determined at least weekly.
- 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

VI. **References**

   [http://dels.nas.edu/ilar_n/ilarjournal/41_2/CancerResearch.shtml]


I. **Purpose** - The purpose of this policy is to provide direction and reference material regarding the production of monoclonal antibodies (MAb) in mice. This policy does not apply to MAb production in other species such as the rabbit.

II. **Introduction** - The production of MAb in mice involves immunizing the animal, selecting antibody producing cells (B cells), fusing the B cells with myeloma cells, creating an ascites-producing hybridoma, and finally injecting hybridoma cells into in primed mice. The NIH concurs with the findings and recommendations in the 1999 Report of the National Research Council Monoclonal Antibody Production which indicates that during the accumulation of ascites fluid there is likely to be pain and distress, particularly when some cell lines that are tissue-invasive are used, and in situations of significant ascites development. The report concludes that there is, and will continue to be, scientific necessity for this method. However, as tissue-culture systems are further developed, tissue-culture methods for the production of monoclonal antibodies should be adopted as the routine method, unless there is a clear reason why they cannot be used. Accordingly, Institutional Animal Care and Use Committees (IACUCs) are expected to critically evaluate proposed uses of the mouse ascites method by investigators.

Prior to approval of such protocols, IACUCs must determine that:
- the proposed use is scientifically justified
- methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered, and
- the latter have been found unsuitable.

III. **Responsibilities** – This document applies to anyone at Rutgers University producing monoclonal antibodies in research rodents.

IV. **Definitions** - none

V. **Methods**

a. **In vitro methods** - *In vitro* methods must be considered first. Refer to the below list for commercial sources for in vitro production of monoclonal antibodies.
   i. **Covance Research Products**: [http://covance.com](http://covance.com)
   ii. **Taconic Biotechnology**: [http://www.taconic.com](http://www.taconic.com)
   iii. **Cell Essentials, Inc**: [http://www.cell-essentials.com](http://www.cell-essentials.com)
b. *In vivo* antibody production

i. **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 document *Fluid Administration and Collection in Rodents* regarding proper needle size and injection volumes.

ii. **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 pristine; ≤0.20 ml should be delivered.

iii. **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.

iv. **Ascites** - The cranial displacement of the diaphragm due to ascites is associated with dyspnea, orthopnea, or tachypnea. It is therefore reasonable to assume that mice with large accumulations of ascites fluid experience discomfort and distress. There is a limit of 3 abdominal taps per animal (two taps in live animals and a final tap after euthanasia); an 18-22 gauge needle should be used. 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.

v. **Clinical Signs** - Animals must be observed for signs of distress and pain. Clinical signs include: rapid or labored breathing, pallor, hunched posture, inactivity, dehydration, inappetence, low body condition score (BCS), rough hair coat, ambulation difficulty, constipation, or diarrhea. Animals that show signs of excessive distress or appear debilitated after any of the taps should be given fluids or euthanized. The Comparative Medicine Resources (CMR) Veterinary Staff must be contacted for immediate evaluation.

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

vii. **Humane Endpoints** - Animals must be euthanized when the following symptoms are observed: prolonged [inappetence, inactivity, diarrhea/constipation, hunched posture, rough coat], hypothermia, tachypnea, labored breathing, pallor, inability to remain upright, or any other clinical signs indicated in *Humane Endpoints* document or CMR Veterinary Staff recommendation.

viii. **Summary of Ascites Production**

<p>| Fluid volume, site of injections, needle sizes | Fluid Administration / Collection document |
| Testing cell lines for murine viruses | Cell Line Use document |
| <strong>Priming</strong> | pristane, 0.20 ml IP |
| <strong>Needle size for tap</strong> | 18-22 gauge |</p>
<table>
<thead>
<tr>
<th><strong>Number of taps</strong></th>
<th>Maximum of three (3\textsuperscript{rd} after euthanasia)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluid volume administered</strong></td>
<td>Fluid Administration / Collection document</td>
</tr>
<tr>
<td><strong>Monitoring after hybridoma inoculation</strong></td>
<td>3 times a week during the first week, then daily</td>
</tr>
<tr>
<td><strong>CFA use</strong></td>
<td>Need scientific justification (only one CFA injection per animal), CFA document</td>
</tr>
<tr>
<td><strong>Fluid replacement after ascites harvesting</strong></td>
<td>1-2 ml warm saline SC</td>
</tr>
<tr>
<td><strong>General anesthesia during tap</strong></td>
<td>Recommended to prevent pain and distress</td>
</tr>
<tr>
<td><strong>Humane endpoints</strong></td>
<td>Humane Endpoints document</td>
</tr>
</tbody>
</table>

VI. References

a. The 1999 report of the National Research Council Monoclonal Antibody Production


d. Duke University: Guidelines for Monoclonal Antibodies


e. Connell University:

I. **Purpose** - The purpose of this document is to provide investigators with information regarding the proper testing of certain products to be introduced into live rodents. This is part of the rodent health surveillance program at Rutgers University, created to prevent the introduction of infectious diseases into animal use facilities.

II. **Introduction** - Cell lines, tissues, and body fluids that have been derived from or passed through rodents can 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 biologic materials can be sources of both murine and human viruses that can contaminate rodents or pose serious risks to laboratory personnel. Rapid and effective assays are available to monitor microbiologic contamination and must be considered before introducing such material into animals.

III. **Responsibilities** – This document applies to any research group at Rutgers University using any of the following products included in section V.a of this document.

IV. **Definitions**

   a. IMPACT - Infectious Microbe PCR Amplification Test

V. **Methods**

   a. **Products requiring testing regardless of source:**

      i. All cell lines of rodent origin obtained from sources that have not been tested for and documented free of murine pathogens that are being administered to rodents (mouse, rat, hamster) at Rutgers.

      ii. Any cell lines passaged through rodents, including human cell lines. **Human cell lines need to be registered with the Institutional Biosafety Committee (IBC) before use in animals.**

      iii. Rodent body fluids (blood and serum), cells, and tissues obtained from sources that have not been tested for and documented free of murine pathogens and intended for use in rodents at Rutgers University. This includes rodent sera for use in cell cultures.

      Note: agents must be tested **before** introducing into the animals.
b. Agents to be excluded

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

<table>
<thead>
<tr>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma spp.</td>
</tr>
<tr>
<td>Mycoplasma pulmonis</td>
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<tr>
<td>Sendai virus</td>
</tr>
<tr>
<td>Mouse hepatitis virus</td>
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<tr>
<td>Minute virus of mice</td>
</tr>
<tr>
<td>Mouse parvovirus (MPV1-5)</td>
</tr>
<tr>
<td>Theiler's murine encephalomyelitis virus</td>
</tr>
<tr>
<td>Murine norovirus</td>
</tr>
<tr>
<td>Reovirus 3</td>
</tr>
<tr>
<td>Mouse rotavirus</td>
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<tr>
<td>Ectromelia virus</td>
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<tr>
<td>Lymphocytic choriomeningitis virus</td>
</tr>
<tr>
<td>Polyoma virus</td>
</tr>
<tr>
<td>Lactate dehydrogenase-elevating virus</td>
</tr>
<tr>
<td>Mouse adenovirus (MAD1, MAD2)</td>
</tr>
<tr>
<td>Hantaan virus</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma spp.</td>
</tr>
<tr>
<td>Mycoplasma pulmonis</td>
</tr>
<tr>
<td>Pneumonia virus of mice</td>
</tr>
<tr>
<td>Kilham's rat virus</td>
</tr>
<tr>
<td>Toolan's H1 virus</td>
</tr>
<tr>
<td>Rat parvovirus</td>
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<tr>
<td>Lymphocytic choriomeningitis virus</td>
</tr>
<tr>
<td>Rat cytomegalovirus</td>
</tr>
<tr>
<td>Sendai virus</td>
</tr>
<tr>
<td>Rat coronavirus</td>
</tr>
<tr>
<td>Sialodacryoadenitis virus</td>
</tr>
<tr>
<td>Rat minute virus</td>
</tr>
<tr>
<td>Seoul virus</td>
</tr>
<tr>
<td>Mouse adenovirus</td>
</tr>
<tr>
<td>Reovirus 3</td>
</tr>
<tr>
<td>Rat theilovirus</td>
</tr>
</tbody>
</table>
c. **Recommended Testing Laboratories:**

(i) **IDEXX RADIL Lab Animal and Biological Materials Diagnostic Testing**

email: idexx-radil@idexx.com  
Phone: 800-669-0825; 573-499-5700  
Fax: 573-499-5701

Mice - IMPACT Profile I (20 agent test)  

Rats - IMPACT Profile V (16 agent test)  

(ii) **Charles River Laboratories** Cell Line / Research Biologics Screening  
Phone: 1.877.CRIVER.1 (1.877.274.8371)

Mouse Essential Panel or Rat Essential Panel  

Other laboratories may be used, but must be pre-approved by the CMR Director or Associate Director of Veterinary Services.

d. **Reporting**

Information on the proposed use of rodent cell lines/biologicals must be provided on the Institutional Animal Care and Use Committee (IACUC) application. Copies of test results must be submitted to the Comparative Medicine Resources (CMR) Director or Associate Director for review and approval, prior to the actual use of said products. Addition of new cell line(s) or biological products for ongoing approved projects also requires testing, review, and approval prior to use.

VI. **References**


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

II. **Introduction**

Procedures involving animals, including the use of drugs, medical materials, anesthetics, analgesics and euthanasia agents are regulated by the Office of Laboratory Animal Welfare (OLAW), the U.S. Department of Agriculture (USDA), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy), and the Animal Welfare Act (AWA, 9 CFR, §2.33) “Attending Veterinarian and Adequate Veterinary Care”, and detailed in USDA Policy #3 “Veterinary Care” (Expired Medical Materials and Pharmaceutical-Grade Compounds in Research).

Rutgers University Comparative Medicine Resources (CMR) enforces policies mandated by the above noted agencies as well as the Rutgers veterinarians’ recommendations that are based on these agency mandates and personal experience.

III. **Responsibilities** – This document applies to all laboratory animal users at Rutgers University.

IV. **Definitions**

a. For the purpose of this document, “**substance**” is defined as any drug, pharmaceutical, medication, antibiotic, experimental and non-experimental food additive or supplement, physiological solution, biological, or chemical.

b. For the purpose of this document, “**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.

c. **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).

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. As a rule, pharmaceutical grade drugs are distributed by veterinary or human medical supply houses (e.g. Henry Schein, Patterson Veterinary Supply) whereas chemical grade drugs are sold by chemical companies such as Sigma-Aldrich.

d. **Chemical (Non-Pharmaceutical) Grade (NPG) Substances** are substances that are not tested by the USP by specific assay methods and product specifications 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.

e. **(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.

https://www.avma.org/KB/Policies/Pages/Compounding.aspx

f. **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 whether they have relative abuse potential or likelihood of causing dependence.

g. **Expired Drugs** are not usable beyond the date included on the package. If only a month and year are provided, the material expires the first day of the next month.

V. Methods

a. **Preparation, Use, Storage and Labeling of Substances and Materials**

i. **Preparation of Substances and Materials** - Substances must be prepared according to manufacturer instructions and any compound-specific guidelines issued by a CMR veterinarian.

ii. **Use of Substances and Materials** - Substances and materials are permitted for animal experimentation, teaching or training provided there is current IACUC approval that describes the use of the substances and materials in the IACUC application and the substances and materials are administered only to the animals described in the IACUC protocol.

iii. **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.
iv. Labeling Aliquots of Substances

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 (drug) including active ingredient(s)
- Date of preparation, dispensing, or transfer to secondary storage vessel
- Quantity originally transferred into secondary storage vessel
- Expiration date (typically less than one month after preparation)
- Concentration(s) of drug(s)
- Contact information: full name of Principal Investigator and telephone number
- All cautionary statements related to proper handling, storage and use

The use of Falcon tubes and Eppendorf tubes is not recommended for mixing and storing substances/aliquots for injection, as it is difficult to maintain sterility when accessing drugs.

b. Non-Pharmaceutical Grade Compounds (Chemical Grade)

“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 should 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 should 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.”

- p31, Guide 2011

Regulatory guidelines require that when available, researchers must utilize pharmaceutical grade drug formulations (i.e. FDA-approved human or veterinary drugs) in lieu of chemical grade drugs. Acute or non-survival procedures are not exempt from this requirement.

Most common indications for chemical grade drugs include -
- Lack of acceptable/available veterinary or human pharmaceutical-grade compounds
- Investigation of novel therapeutic drugs

Cost savings is not an adequate justification for using non-pharmaceutical-grade compounds.

When using chemical grade compounds -
- Must be described in an animal use protocol approved by a Rutgers IACUC.
- 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 outside contaminants. The use of Falcon tubes is not recommended for mixing and storing substances for injection, as it is difficult to maintain sterility when accessing drugs.

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

http://www.pattersonvet.com/Supplies/ProductFamilyDetails/PIF_345703
https://www.fishersci.com/shop/products/depyrogenated-sterile-empty-vials/p-4526493

• Diluents or vehicles must be specified in the animal use protocol. Use of solvents (e.g. ethanol, acetone, benzene, carbon tetrachloride, dimethylformamide, etc.) will be evaluated on a case-by-case basis. Use of such solvents may limit amounts, concentration, and routes of administration.
• Containers (e.g. injection vial) must be labeled with the drug, concentration, date of preparation, and date of expiration.
• 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.
• 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. An exception to this is a cocktail of ketamine and xylazine which may be kept for up to 6 months. Solutions must not be used if they are cloudy, discolored, precipitated or otherwise altered.
• pH of solutions must be between pH 4.5 and 8.0. Use of a solution with a pH outside this range must be addressed in the animal use protocol.
• Pyrogens are compounds such as endotoxins which may cause fever when injected into an animal. All pharmaceutical drugs are tested for pyrogens. Sterility does not assure that pyrogens are not present. Filtering does not remove pyrogens. Pyrogen testing is not practical for small lots of prepared drug. Pyrogenicity is a potential experimental variable that researchers should be aware of when using non-pharmaceutical grade drugs.

Acceptable solvents (sterile filtered if possible):
• Distilled water
• PSS (0.9% NaCl), PBS, balanced salt solution (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) dimethylsulphoxide (DMSO)
• Cyclodextrins5 (e.g. 2-hydroxypropyl-beta-cyclodextrin, Trappsol®)

c. Approved Uses of Euthanasia Grade Sodium Pentobarbital as an Anesthetic

Pharmaceutical grade pentobarbital for injection (Nembutal) is currently very difficult to acquire and frequently out of stock from vendors without reasonable availability dates being provided. Recent
communication with regulatory agencies has confirmed that the use of euthanasia grade pentobarbital as an anesthetic is acceptable under the following research conditions:

- Perfusion under anesthesia
- Exsanguination under anesthesia

“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”

**Euthanasia grade pentobarbital may not be used for any survival anesthetic procedures.**

Investigators must evaluate each product with respect to how ingredients might affect their research. Examples of euthanasia grade pentobarbital formulations include:

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>DEA Schedule</th>
<th>Pentobarbital mg/ml</th>
<th>Phenytoin* mg/ml</th>
<th>Isopropyl alcohol</th>
<th>Ethyl alcohol</th>
<th>Propylene glycol</th>
<th>Benzyl alcohol</th>
<th>Dye</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthasol®</td>
<td>Virbac</td>
<td>CIII</td>
<td>390</td>
<td>50</td>
<td>-</td>
<td>10%</td>
<td>18%</td>
<td>2%</td>
<td>Rhodamine B</td>
<td>100</td>
</tr>
<tr>
<td>Beuthanasia®</td>
<td>Intervet/Merck Animal Health</td>
<td>CIII</td>
<td>390</td>
<td>50</td>
<td>-</td>
<td>10%</td>
<td>18%</td>
<td>2%</td>
<td>Rhodamine B</td>
<td>100</td>
</tr>
<tr>
<td>Euthanasia-III Solution®</td>
<td>Med-Pharmex</td>
<td>CIII</td>
<td>390</td>
<td>50</td>
<td>-</td>
<td>10%</td>
<td>18%</td>
<td>2%</td>
<td>Rhodamine B</td>
<td>100</td>
</tr>
<tr>
<td>Euthanasia Solution®</td>
<td>Vedco</td>
<td>CIII</td>
<td>390</td>
<td>50</td>
<td>-</td>
<td>10%</td>
<td>18%</td>
<td>2%</td>
<td>Rhodamine B</td>
<td>100</td>
</tr>
<tr>
<td>Somnasol®</td>
<td>Henry Schein™ Animal Health</td>
<td>CIII</td>
<td>390</td>
<td>50</td>
<td>-</td>
<td>10%</td>
<td>18%</td>
<td>2%</td>
<td>Rhodamine B</td>
<td>100</td>
</tr>
</tbody>
</table>

**COMPARISON**

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>DEA Schedule</th>
<th>Pentobarbital mg/ml</th>
<th>Phenytoin* mg/ml</th>
<th>Isopropyl alcohol</th>
<th>Ethyl alcohol</th>
<th>Propylene glycol</th>
<th>Benzyl alcohol</th>
<th>Dye</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nembutal</td>
<td>Lundbeck Inc.</td>
<td>CII</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>10%</td>
<td>40%</td>
<td>-</td>
<td>-</td>
<td>20, 50</td>
</tr>
</tbody>
</table>

*Phenytoin sodium produces toxic signs of cardiovascular collapse and/or central nervous system depression.

**d. Expired Substances and Materials**

The use of expired substances is not consistent with acceptable veterinary practice or adequate veterinary care and is strictly forbidden for use in live research animals. Euthanasia, anesthesia and analgesia agents cannot be used beyond their expiration date, even if a procedure is terminal.

Expired materials can only be used in non-survival studies (see Rodent and Non-Rodent Surgery IACUC documents for more details). All expired materials must be clearly labeled ‘EXPIRED – ACUTE USE ONLY’ and stored physically separately from non-expired materials.

Expired materials should not be used unless the manufacturer verifies efficacy beyond the expiration date, or the investigator is able to document to the satisfaction of the IACUC that such use would not negatively impact animal welfare or compromise the validity of the study. The veterinarian and IACUC must maintain control over the use of expired medical materials in order to meet their responsibilities to avoid or minimize discomfort, pain or distress to animals.

Expired substances and materials should be promptly discarded in accordance with Rutgers Environmental Health & Safety (REHS) policy. If not discarded, expired drug containers must be labeled “expired” and stored separate from drugs in use. Controlled substances cannot be discarded.
without appropriate paperwork. All controlled substances must be stored in an approved secure cabinet or safe.

Members of the IACUC can 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.

e. 2,2,2 Tribromoethanol (TBE) / Avertin

Tribromoethanol is an injectable anesthetic agent that was once manufactured as a pharmaceutical grade drug under various trade names, Avertin is the best known. Pharmaceutical grade tribromoethanol is no longer available and investigators who wish to use this anesthetic must make their own solutions with non-pharmaceutical grade tribromoethanol compounds.

Investigators must adhere to the following tribromoethanol guidelines:

- Because alternative, pharmaceutical grade anesthetics are available, scientific justification for the use of tribromoethanol must be included in all protocol and amendment applications submitted to the IACUC.
- Tribromoethanol 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.
- Diluted tribromoethanol must be stored at 4°C (39°F) and protected from light to prevent degradation. 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.
- If the solution is less than pH 5, it should be presumed to have degraded.
- If the solution develops an unusual discoloration (typically yellow) or forms a precipitate, the solution should also be discarded.

The side effects of tribromoethanol include acute inflammatory changes, local irritation, fibrous adhesions in the abdominal cavity, and death following one or repeated IP injections.

f. Controlled Substances (CS)

Rutgers Controlled Substances Program – Rutgers University has a centralized Controlled Substances Program. For more information regarding this program, please access the following link:

Record of use - The use/administration of controlled substances must be recorded every time the drug is administered to an animal. When a controlled drug is incorporated into a drug cocktail (such as ketamine/xylazine, common in rodents) or is diluted with saline or other diluent, the initial amount of controlled drug used to create the cocktail/dilution must be recorded, then an additional log sheet created for the cocktail/dilution. On the vial include date of creation, expiration date, concentration of each component, and dosing information. Record individual administration of cocktail/dilution volume on the log sheet. Once the drug or cocktail has been consumed or expired, all records of use must be given to the appropriate CS Unit Coordinator (varies by campus and location). Copies can be made for laboratory/experimental records.
Storage – Controlled substances must be secured within a locked steel cabinet, drawer, or safe that cannot be moved or transported (if less than 750lbs). The storage cabinet must be kept in a room within the laboratory that can be locked as well, ensuring a two-lock system.

Access to controlled substances stored within laboratories or other procedure areas is the responsibility of the Principal Investigator. Keys to storage boxes or drawers must be maintained in a secure area such that the key(s) is only accessible to the designated personnel. Keys are never permitted to be left in the lock or on a shelf or drawer near the actual CS storage location. Lab staff who don’t utilize CS should not have access to CS. The list of lab personnel with access to CS should be provided to the CMR staff who oversees the CS program.

General guidelines -
- Store all drugs in a secure, dedicated location
- Assign responsibilities to one specific individual, with another individual as backup
- Establish an inventory system that minimizes the amount of drug or medical supplies on hand
- Perform regular monthly checks of inventory and discard all expired drugs or medical materials following REHS policies.

Additional information regarding CS can be found in the Anesthesia and Analgesia in Laboratory Animals IACUC document.

VI. References


b. Animal Welfare Act: Code of Federal Regulations: 9 CFR Chapter 1 Subchapter A, Parts 1, 2 and 3


g. http://grants.nih.gov/grants/olaw/faqs.htm#f4


i. Cyclodextrins: Improving Delivery of Hydrophobic Compounds, Alzet,


I. **Purpose** - The purpose of this policy is to describe the specialized care of Experimental Allergic Encephalomyelitis (EAE) animals in order to ensure their humane care and treatment.

II. **Introduction** - EAE is an animal model for central nervous system autoimmune disease and is widely used as a human Multiple Sclerosis (MS) model. Although clinical signs vary according to species and strain, they typically include visual, sensory, and motor deficits. This generally manifests as an ascending paralysis graded on a five-point scale ranging from the loss of tail tone in an otherwise normal animal (1) to one in a moribund condition (5). The course may vary from one or more episodes with short periods of remission of clinical signs, to a progressive, chronic state.

III. **Responsibilities** – This document applies to any Principal Investigator or associated laboratory staff using either an induced (i.e. CFA) or spontaneous EAE model.

IV. **Definitions** - none

V. **Methods**

**Care of EAE mice:**

1. Every animal to be injected with any substance designed to elicit EAE must be identified with a cage card containing the letters “EAE” (can be handwritten on existing cage card(s)).
2. At the time of inoculation, post-procedural cage card specifying “EAE mice” and date of inoculation must be placed on every applicable cage.
3. Once animals develop clinical signs, animals must be monitored at least daily, including weekends and holidays.
4. Food must be placed on the floor of the cage and a water bottle with a long sipper tube must be used. An alternative source of water should be provided (e.g. Napa Nectar or Hydrogel).
5. Monitoring includes the graded score of EAE development (see Table 1), hydration status, body condition score, general condition, and activity level of the animal(s). All observations and treatments must be recorded on the post-procedural cage card, dated, and initialized.
6. All paralyzed mice should be monitored for skin irritation associated with urine scalding and, if male, observed for penile irritation secondary to flaccid paralysis.
7. Paralyzed animals should be removed from the cage if healthy mice compromise their health. Healthy animals may walk on paralyzed animals, causing discomfort and/or injuries, and may eat food intended for paralyzed animals.

**Humane endpoints (euthanasia indicated):**

- EAE grade 4 and 5 (see EAE clinical signs and interventions table below for exceptions)
- BCS ≤1.5 or prolonged body weight loss ≥30%
- Veterinarian recommendation
### EAE Clinical signs and Interventions

| N/A       | Normal                                                                 | 1. Baseline weight  
|           |                                                                       | 2. Write "EAE" on record cage card  
|           |                                                                       | 3. Write immunization date  
| early     | Tail tone: Decreased tail tone                                         | 1. Start record keeping (activity, EAE stage #)  
|           |                                                                       | 2. Monitor daily  
| early     | Hind limb Paresis: Weakness in hind limbs, animals have difficulty to move, appear ataxic or "clumsy" | 1. Monitor BW 1X a week and record  
|           |                                                                       | 2. Use long sipper tubes, soft food or gel diets  
|           |                                                                       | 3. Provide moistened food pellets on cage floor  
| middle    | Hind limb paralysis: Inability to move one or both hind limbs         | 1. Follow stage 2  
|           | Urinary Incontinence: Urine is leaking, urine scald around prepuce or vulva | 2. Palpate bladder 3X/week  
|           | Dehydration: Decreased skin turgor                                    | 3. Monitor for skin lesions, urine scald, penile prolapse  
|           | Oral/lingual paralysis: Inability to swallow                           | 4. Use SC fluid for dehydration  
|           |                                                                       | 5. Use softer bedding materials (such as alphadry)  
|           |                                                                       | 6. Provide food via oral gavage if animal unable to swallow  
| middle to late | Weakness of fore limbs with paraparesis or quadriparesis              | 1. Follow stage 3  
|           | Atonic bladder: Enlarged, unable to urinate                           | 2. For atonic bladder, express bladder at least 2X/day.  
|           | Dehydration: Decreased skin turgor                                    | 3. Monitor for skin lesions, urine scald, penile prolapse  
|           | Oral/Lingual paralysis: Inability to swallow                           | 4. Use SC fluid for dehydration  
|           |                                                                       | 5. Use softer bedding materials (such as alphadry)  
|           |                                                                       | 6. Provide food via oral gavage if animal unable to swallow  
| late end stage | Quadriplegia: Paralysis of all four limbs                              | 1. Follow stage 4  
|           | Atonic bladder: Enlarged, unable to urinate                           | 2. Animals must be euthanized unless exempted by the IACUC  
|           | Dehydration: Decreased skin turgor                                    | 3. All moribund, body weight loss ≥ 30%, dyspneic animals must be euthanized  
|           | Oral/lingual paralysis: Inability to swallow                           | 4. Use SC fluid for dehydration  
|           | Dyspnea: Difficulty or abnormal breathing                             | 5. Use softer bedding materials (such as alphadry)  
|           | Moribund: Animal is not moving, recumbent                             | 6. Provide food via oral gavage if animal unable to swallow  

### Table 1- Clinical grading of EAE mice

<table>
<thead>
<tr>
<th>EAE GRADE</th>
<th>CLINICAL SIGNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal mouse; no overt signs of disease</td>
</tr>
<tr>
<td>1</td>
<td>Decreased tail tone or weak tail only</td>
</tr>
<tr>
<td>2</td>
<td>Hind limb weakness (paraparesis)</td>
</tr>
<tr>
<td>3</td>
<td>Hind limb paralysis (paraplegia) and/or urinary incontinence</td>
</tr>
<tr>
<td>4</td>
<td>Weakness of fore limbs with paraparesis or paraplegia (quadriparesis) and/or atonic bladder</td>
</tr>
<tr>
<td>5</td>
<td>Paralysis of all limbs (quadriplegia), moribund state; death by EAE</td>
</tr>
</tbody>
</table>
VI. References


I. Purpose – In general, photography of research animals is not permitted except as outlined in this document. This document provides guidance and responsibilities in handling requests by 1) visitors to take photographs and video of laboratory animals in animal facilities, 2) Principal Investigators and laboratory members using photographs and video for research purposes, and 3) Comparative Medicine Resources (CMR) staff using photography for clinical purposes. It is intended to protect the confidentiality and integrity of research and to provide an accurate representation of research at Rutgers University.

This document does not apply to the use of security cameras placed in animal facilities to monitor animal and human safety.

II. Introduction – In the current climate of aggressive animal rights groups protesting use of laboratory animals in the university setting, it is critical that all photographs and video recordings of animals used in the context of research are portrayed accurately and fairly. There is a legitimate need for photography of laboratory animals at Rutgers University to document clinical disease, staff non-compliance, research practices pertaining to publishing of studies (both for data collection and procedural documentation), and potential documentation by outside regulatory agencies.

III. Responsibilities – This document applies to the three scenarios listed in section I, investigators performing field research (field studies - See Field Studies document for further details), along with any other situations identified by CMR veterinarians.

IV. Definitions

a. Animal Facility: Any CMR-operated animal holding area (aka vivarium). This does not include the Rutgers School of Environmental and Biological Sciences (SEBS) agricultural facilities on the Cook Campus farm.

b. Animal Research Laboratory or Procedure Area: Any room or area outside of a Rutgers Animal Facility but on a Rutgers University campus where animals are used in research, testing, or training.

c. 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).

d. Recording: Any photograph, file, image, tape, or video created by a Device.

e. Visitor: Any individual without personal key or keycard access to a specific Animal Facility or individual visiting an Animal Research Laboratory or Procedure Area who is not described in an Institutional Animal Care and Use Committee (IACUC) protocol specific to that location.
Visitors do not include employees, fellows, or students of the University authorized to participate in the specific IACUC-approved protocol; consultants or other non-Rutgers University faculty, staff, students, outside vendors, or University employees acting as an authorized agent and approved for work on a specific IACUC approved protocol. See Visitors to Rutgers Animal Facilities document for further details.

V. Methods

a. General considerations for recording
   - Recording devices must be sanitized prior to Vivarium entry according to Vivarium recommendation. Contact Area Supervisor for further details.
   - Recordings must show appropriate and accurate context (e.g. if an animal is anesthetized or sedated, include the vaporizer or tray holding the bottle of injectable drug).
   - Appropriate personal protective equipment must be worn by all persons in the recording.
   - All attempts must be made to have animals in clean surroundings - clean cages or clean pens with clean accessories. Water bottles and feeders must be full if visible in the recording.
   - All IACUC approved policies, SOPs, and guidelines must be followed.
   - No references to personal information can be visible. Pay attention to background and items such as cage cards.
   - Recordings of personnel require approval of each individual recorded.
   - Appropriate handling and restraint methods for the species must be used.
   - The smallest portion of the animal, surface, or room must be shown whenever possible.
   - Recordings must be downloaded into a secure computer and deleted from the device before leaving the University.
   - Encrypted file transfer must be used for recording dissemination.
   - Recordings are not allowed to be posted on any social media sites.

b. Research Staff
   - All procedures shown must be described in the approved IACUC protocol for that particular animal.
   - No animals that are ill, have visible lesions, or visible research alterations (implants, tumors, etc.) are to be photographed unless approved by the CMR Director or Associate Director and the photography is required for scientific publication and/or data analysis by the PI.
     - Exception: Research staff is permitted to send recordings directly to a veterinarian for purposes of clinical evaluation without prior approval. Original recording must be destroyed/deleted once received by the veterinarian.

   c. CMR Veterinary Staff
      - Recordings are for educational seminars, clinical diagnoses, documentation of non-compliance, or post-approval monitoring (PAM) only.
      - Recordings must be downloaded onto a secure computer and distributed only by encrypted email.
      - Recordings used for training purposes must not have any reference to the PIs or the facility.
      - When performed by animal care staff, an animal care manager, supervisor, or member of the veterinary staff must be present for all images being taken.
d. Visitors

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 recording is not allowed except for official purposes that are approved by the CMR Director or Associate Director, or the Director of the Office of Research Regulatory Affairs. Recordings of animals inside farm buildings must be approved by the farm manager.

- Visitors are not permitted to take recordings in the animal facility except: (1) government inspector and photodocumentation is necessary for official duties or (2) visitor is serving as a photography vendor for the faculty - all such vendor photodocumentation is subject to the points listed above and must be approved by the CMR Director or Associate Director. Only IACUC approved procedures can be recorded.
- The CMR 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.
- An animal care manager, supervisor, or member of the veterinary staff must be present for all recordings being taken.

When performed by government inspectors (e.g. USDA Veterinary Medical Officer) or private inspectors (e.g. AAALAC Site Visitor) conducting a site inspection who elect to take recordings, the visitor must agree to the following conditions:
- Recordings can be taken only as an official part of the inspection.
- Recordings used in a report must be provided to CMR Director or Associate Director for review and approval, as are all other components of site inspection reports.
- All negatives, prints, and images not used in an official report must be destroyed within 48 days.
- Recordings will not be distributed or used in any way other than as supporting evidence for an official site inspection report.
- 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.
- USDA Veterinary Medical Officers must allow the Institutional Official to review or redact the records for proprietary business information, research facility records, protocols, or IACUC minutes. The inspector must allow the facility 24 to 48 hours for this purpose (Animal Welfare Inspection Guide Required Inspection Procedures v. 03-25-13, section 2-7, 2-8).

**Note:**
The CMR Director and or Associate Director reserve the right to review all recordings before release, and may require that these recordings be destroyed.

VI. References - none.
I. Purpose - Under special circumstances it may be appropriate to house animals outside of centralized animal care facilities. This document outlines the conditions under which this is appropriate and establishes guidelines to be met by investigators so that the Institutional Animal Care and Use Committee (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 animal facilities.

IV. Definitions – A satellite animal facility is defined as an animal holding area outside of a Comparative Medicine Resources (CMR) vivarium for greater 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 advance by the IACUC as part of an approved animal use 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
• Instances where the investigator has an established housing system in place, which is suitable and may be expensive or difficult to move

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 will be negotiated between the PI and CMR.

c. Satellite Requirements

i. A satellite animal facility must have all 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 entry access to the satellite facility at all times (24 hours a day). If surgery is planned, appropriate space and equipment must be provided.

ii. All animals must be observed at least once daily, 365 days per year. Observations must be documented each day at the time of observation and documentation must be available for review by CMR staff or the IACUC at any time.

iii. 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.

iv. If 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.

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

d. Termination of a Satellite Facility

i. If this policy is not followed, use of a satellite animal facility is subject to immediate termination by the IACUC. In case of termination, the satellite facility may be run by the CMR at a minimum of twice standard per-diem rates, until the research is completed.
ii. 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


b. Animal Welfare Act

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Light</td>
<td>dark/light 12/12 automatic timer</td>
</tr>
<tr>
<td>2 Temperature</td>
<td>accepted range 18-25°C (68-79°F)</td>
</tr>
<tr>
<td>3 Humidity</td>
<td>between 30-70%</td>
</tr>
<tr>
<td>4 Feed / bins</td>
<td>vermin-proof containers, sanitize container(s) every 2 weeks</td>
</tr>
<tr>
<td>5 Water</td>
<td>chlorinated reverse osmosis or acidified</td>
</tr>
<tr>
<td>6 Room Air</td>
<td>minimum 10-15 fresh-air exchanges/hour, keep animal room doors closed</td>
</tr>
<tr>
<td>7 Enrichment</td>
<td>Social housing, enrichment objects in each cage</td>
</tr>
<tr>
<td>8 Animal manipulation</td>
<td>performed under BSL-2 hood for allergy prevention and biosecurity</td>
</tr>
<tr>
<td>9 Vermin control</td>
<td>humane mouse trap in room, log of daily trap monitoring</td>
</tr>
<tr>
<td>10 Cage changing</td>
<td>static cages weekly, ventilated cages bi-weekly or more as needed</td>
</tr>
<tr>
<td>11 Animal monitoring</td>
<td>daily (including weekend and holidays) or more as indicated in the IACUC</td>
</tr>
<tr>
<td></td>
<td>protocol, daily log must be kept and available at all times</td>
</tr>
<tr>
<td>12 Biohazard</td>
<td>follow REHS recommendations</td>
</tr>
<tr>
<td>13 Disaster plan</td>
<td>portable heater, cooling, back-up power, feed/water provisions</td>
</tr>
<tr>
<td>14 IACUC documents</td>
<td>Must follow all applicable documents - e.g., rodent enrichment, cage density, humane endpoints, food / water regulation</td>
</tr>
</tbody>
</table>

Note – Individual Rutgers campuses and facilities may have additional requirements for satellite facilities based on location
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 immersion 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 used multiple times in the same animal safely.

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

III. **Responsibilities** – This document applies to anyone at Rutgers University who is injecting adjuvant(s) into live research animals.

IV. **Definitions** - none

V. **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 aseptically prepared, including shaving of site followed by disinfection with surgical scrub.

**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 USDA Category E
- CFA is only allowed to be administered to each animal once (usually initial immunization)
- CFA should be prepared 1:1 (volume) with aqueous 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 CFA-antigen emulsion administration (all volumes in microliters, mls):**

<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>ID</th>
<th>IP</th>
<th>FP</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>&lt;0.1</td>
<td>*</td>
<td>&lt;0.2**</td>
<td>&lt;0.05***</td>
<td>&lt;0.05***</td>
</tr>
<tr>
<td>Rat</td>
<td>&lt;0.1</td>
<td>&lt;0.05**</td>
<td>&lt;0.5**</td>
<td>&lt;0.1**</td>
<td>&lt;0.1***</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;0.25</td>
<td>&lt;0.05**</td>
<td>*</td>
<td>*</td>
<td>&lt;0.25***</td>
</tr>
</tbody>
</table>

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 for Rodent Monoclonal Antibody Production

**VI. References**


I. **Purpose** - Rutgers University is committed to the humane treatment of all animals used in research, testing, teaching and production. The privilege to use live animals for the advancement of science and medicine carries with it the responsibility to follow all applicable laws, policies, and procedures concerning animal welfare, as developed by the government and Rutgers University. 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, and employees of Rutgers University

IV. **Definitions** - none

V. **Methods**

Rutgers University is committed to the humane care and use of all animals used for teaching and research. If you see or know of activities which you believe constitute inappropriate animal care or use, you are encouraged to report such activities. Inappropriate care and use may include inhumane treatment, abuse, neglect, unapproved procedures, etc. The Institutional Animal Care and Use Committee (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 Animal Welfare Act prohibits discrimination or reprisal against any person for reporting violations of any regulation or standards under the Act.

All reports to the CMR staff and/or the IACUC will remain confidential.

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:

Comparative Medicine Resources: Executive Director, Associate Director, Veterinarian, Assistant Director of Facilities and Operations, Facility Manager, and Supervisor

Institutional Animal Care and Use Committee: any member including Chair, Vice Chair, Administrator, or Manager

Director of Research Regulatory Affairs

Institutional Official

Any questions or concerns can also be sent to: animalconcerns@rutgers.edu or submitted by telephone to: Rutgers Compliance Hotline: 1-800-215-9664 or online at: www.rutgers-compliance-hotline.com

VI. References


b. The Animal Welfare Act

c. 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
I. **Purpose** - This document covers the procedures to be used by Rutgers University investigators and staff when transporting research animals between Rutgers animal facilities on any campus.

Animal transfers outside the Rutgers system must be performed via an 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 to laboratories 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 clean 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.
- Comparative Medicine Resources (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** - Principal Investigators or designees are responsible for implementation and oversight of these procedures. Note that the *Photography of Laboratory Animals at Rutgers* Institutional Animal Care and Use Committee (IACUC) document applies to all animals that are being transported for any reason.

Food and water must be provided to any animals being transported.

IV. **Definitions**

a. **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.

i. All containers must meet International Air Transport Association (IATA) specifications.
V. Methods

a. Transfer to Other Institutions – Animal export to other institutions is detailed via Import/Export standard operating procedures (SOPs). Animals cannot be transferred to another institution without prior approval/authorization from the receiving institution.

b. Transfer between Rutgers Campuses or Rutgers Animal Facilities – 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 by 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).

Investigators must have an approved IACUC amendment 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. It some cases animal transfers may be denied at the discretion of the CMR Director or Associate Director.

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.

d. Transfer from Animal Facility to Investigator Laboratory

i. Notify CMR managers or supervisors each time animals are removed from the animal facility to be held in a laboratory for periods longer than 12 hours or overnight to secure appropriate log sheets for documentation of animal census, husbandry and health checks. Laboratories that hold rodents greater than 24 hours must have the laboratory listed as a satellite facility in an IACUC approved protocol; see Satellite Animal Facilities document for more information.

ii. Because of public health, animal health, security, and other public relations concerns, 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.

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

iv. Care must be taken to minimize the time spent in common hallways or lobbies when transporting animals between animal facilities and laboratories. Cages and carts must be covered to ensure animals are shielded from public view. Make sure the cover does not interfere with air flow to the cage(s).

v. Turn water bottles around (if applicable) 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.
vi. Efforts must be made to minimize the amount of stress animals may experience during transport.

vii. 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. The preferred location is a fume hood; however no chemicals or other clutter is permitted in the hood while animals are present.

viii. Food and water must be provided to animals in PI labs.

ix. 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.

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

Note: Most 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

i. All rodents originating at Rutgers must be in a CMR approved shipping crate and provided with bedding, food, and a non-vegetative water source such as Hydrogel or Napa Nectar. Secondary containment may be required at the discretion of the veterinary staff.

ii. Animal crates must be discreetly cloaked under a sheet.

iii. Throughout transportation the ambient temperature in the vehicle may not exceed 85°F nor fall below 45°F.

iv. Transportation of live rodents must be in accordance with standards for hamsters and guinea pigs as described in the Animal Welfare Regulations, revised January 1, 2002 (CFR Title 9, Chapter 1 parts 3.35-3.41).

v. Animals must be picked up and delivered at designated loading docks only. Animals cannot be carried on paths, sidewalks, or streets during delivery.

vi. University-Owned Vehicles
   1. Driver must have prior approval by Rutgers University to operate a university-owned vehicle.

vii. Privately-Owned Vehicles
   1. Privately-owned vehicles can only be used if a University-owned vehicle is not available. The vehicle must have adequate climate control to maintain the temperature within the accepted range of 45-85°F at all times.
   2. The CMR Director or Associate Director must pre-approve the use of a private vehicle and driver (if non-CMR staff) for animal transport. The vehicle must be inspected before it can be approved.
   3. Privately-owned vehicles cannot be used if they fail to meet any of the criteria outlined in this document.
4. No stops are allowed except in emergencies once the journey has commenced.
5. Private vehicles cannot be used to transport hazardous animals/cages unless approved by CMR and REHS.

VI. References

a. Animal Welfare Act
I. **Purpose** – The purpose of this document is to procedure a scoring and treatment system for investigators that perform experiments which may result in self-mutilation of 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 process typically occurs after damage to either the central nervous system (primarily the spinal cord and thalamus) or the peripheral nervous system (such as transection or crushing of the sciatic or femoral nerves). While the cause of this condition is not fully understood, it is most likely a result of neuropathic ('phantom') pain. Other diseases such as diabetes, multiple sclerosis, alcoholism, chemotherapy, and viral diseases also have been shown to cause neuropathic pain in humans and rodents.

III. **Responsibilities** - This document applies to any investigator or associated laboratory member that performs experiments that could result in self-mutilation of test subjects.

IV. **Definitions**

   a. **Autotomy** - self-amputation of limbs or digits

   b. **Autophagia** - self-mutilation of a region of the body

V. **Methods**

   There are currently no generally accepted or effective therapies for neuropathic pain in rodents. 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 actually have been shown to exacerbate the condition and prolong recovery.

   **Self-mutilation scoring:**

   Autotomy – ANY loss of the extremity (including single digits) is considered autotomy

   - Any animal that self-amputates must be euthanized immediately (no treatment).
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:
Any animal 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) highly recommends (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 (a). Over the counter pediatric syrup is usually palatable by 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) or home-made formulations can be created to avert/treat the condition (e).

If not treated preemptively, at the first sign of autophagia (score of 1-2), the animal MUST be treated (or euthanized) with oral acetaminophen (Tylenol, 64mg/kg once daily) and outfitted with an E-collar. 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.

VI. References


I. **Purpose** – This document provides assurance that all individuals entering Rutgers University animal facilities are adequately trained and have been risk-assessed for potential health problems associated with animal contact.

II. **Introduction** - There are valid scientific, educational, and institutional reasons that visitors should be permitted to enter animal facilities of Rutgers University. Visitors may enter animal facilities as part of a collaborative scientific effort to demonstrate protocol effectiveness, an educational effort, to assist in fund raising, 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 by unauthorized individuals.

III. **Responsibilities** - Principal Investigators (PI) or designees are responsible for implementing these procedures and notifying the Institutional Animal Care and use Committee (IACUC) and Comparative Medicine Resources (CMR). PIs or designees are also responsible for ensuring all Rutgers Environmental Health & Safety (REHS) safety restrictions and procedures are followed by visitors.

IV. **Definitions**

   a. **Animal Facility** - Any CMR-managed area that houses vertebrate experimental animals of any species. Animal facilities may include animal housing areas, procedure areas, storage rooms, office space, and hallways.

   b. **Laboratories** are spaces where experimental animals are temporarily kept (periods less than 12 hours) and where procedures may be performed consistent with IACUC approved protocols. Access to laboratory areas, including laboratory areas where animals are temporarily kept, by visitors is the responsibility of individual PIs and members of their research teams in accordance with institutional policy.

   c. **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.

   d. **Visitors** include anyone not otherwise authorized 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. For the purpose of this document, a visitor could be a non-Rutgers employee who has a specific scientific or educational purpose to enter an animal facility, observe an animal procedure, or participate in animal
experiments. A visitor could also be a Rutgers University employee, fellow, or student not included as an authorized and trained participant in an IACUC approved protocol. Use of visitor status under this document for such employees, fellows, or students as visitors is intended for single visits only. Rutgers University employees, fellows, or students requiring multiple visits to animal facilities or research laboratories are expected to complete all IACUC-mandated training and to be added to an IACUC approved protocol.

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.

V. Methods

a. Visitors to Animal Facilities

Visitors may be permitted in animal facilities of Rutgers University if approved in advance by the CMR as long as appropriate safeguards are in place to screen visitors for health issues related to animal exposure and the visitor is accompanied at all times by an authorized guide.

Faculty guides must also notify a member of CMR management that they will be sponsoring a visitor. The guide must provide CMR with the visitors’ names, the reason for the visit, the planned visit time, and facility location by e-mail or in writing at least one business day in advance of the visit. For visitors who wish to enter rooms where animals are in residence, notice should be given at least 10 business days in advance so that Occupational Health requirements can be met. However, such advance notice is not a guarantee that occupational health requirements will be met or that facility access will be approved by CMR.

It is the responsibility of the faculty or facility 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 be advised to avoid entering animal facilities while animals are in residence or to take all appropriate precautions to avoid or limit exposure. If there is any question regarding a risk to the visitor’s health, the visit must not occur until such time as the visitor is clearly informed of the risks involved. It is also the responsibility of the faculty or facility guide to discuss with the visitor the risks that he or she may pose to research animals. Persons who have active tuberculosis, influenza, 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 cannot enter a Rutgers animal facility. Any exceptions must be cleared by the Director of CMR or designee beforehand.

- Healthy, adult, non-immunocompromised visitors may enter animal facilities, tour hallways, and view animals through doorways without entering animal housing or procedure areas without prior occupational health screening.

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• Visitors who wish to enter animal housing or procedure areas while animals are in residence 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.

• Visitors who wish to handle animals must successfully complete the IACUC mandated orientation training. All such visitors must become registered with the IACUC as part of the specific protocol under which the animals are registered. Note that such individuals, when meeting these requirements, will no longer be considered visitors under this document (see Definitions).

Visitors must be under the direct supervision of a guide at all times during a tour or visit. In addition to IACUC approved investigators, other individuals who may serve as guides include a member of CMR management (Director, Associate Director, Clinical Veterinarian, Veterinary Technician, Facility Manager or Area Supervisor).

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 or Associate Director and the Senior Vice President for Research and Economic Development. Such a tour generally may only be initiated in consultation with the IACUC.

Pets may not enter animal facilities.

Visitors cannot take still or video photographs in the animal facility unless 1) the visitor is serving as a photography vendor for the faculty and/or CMR guide, 2) the photographs are necessary or allowed for the documentation of work under an IACUC approved protocol, and 3) the original negatives or electronic media are retained as the property of Rutgers University. The faculty member or guide should advise visitors concerning the prohibition of photography in conjunction with any request for a visit. Visitors requesting photographs may commission the University to provide such photographs. See IACUC document Photography of Laboratory Animals at Rutgers for more information.

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 “hands-on” in the procedure. These visitors are governed by all of the points above, plus the following: 

Visitors Observing Animal Procedures: The PI must provide documentation that occupational health requirements for this visitor have been met either through Rutgers’s Occupational Health program, Rutgers’s Student Health Center, or that of another institution if the visitor is from outside of Rutgers. This request will be reviewed by the CMR; after approval, the PI will be notified and the documentation will be placed in the appropriate PI’s correspondence file.
Special considerations may be needed for Rutgers University students who are not employees. Their occupational health requirements may be completed through the Student Health Center, provided that documentation is provided to the CMR.

Individuals Performing Animal Procedures:
Visitors who will perform animal procedures must meet all the same requirements as non-visitors who perform animal procedures. Note that individuals meeting these requirements are no longer considered visitors under this document (see Definitions). The procedure for granting permission to perform animal procedures for these individuals is as follows:

- The individual must provide documentation that occupational health requirements have been met. The fax cover sheet should include the PI’s name, Animal Protocol number, name of the Visiting Researcher, Scholar or Intern, and the specific educational or research purpose for the visitor’s participation in the animal procedure.
- The individual must complete the IACUC-mandated training requirements.
- Once Occupational Health and training requirements have been met, the visitor must be added to the relevant protocol by the PI.

c. Minor Visitors to Animal Facilities or Laboratories

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:
- No one under the age of 15 is allowed to enter animal facilities
- Some facilities may exclude all minors with no exceptions

Minors who wish to observe animal research must meet the following requirements in full:
- The Institutional Policy on Minors in Laboratories has been read and signed by the faculty member/researcher with responsibility for activities in the laboratory
- The minor’s parent or legal guardian has read and signed a Consent for a Minor in Laboratories
- The minor has completed safety training approved by the Rutgers Environmental Health and Safety Office
- The minor is directly supervised by the faculty member/researcher or designated supervisor at all times while in the laboratory
- A Risk Management registration form for minors present in laboratories that describes the educational objective of the experience has been completed.

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

<table>
<thead>
<tr>
<th>Description of Visitor(s)</th>
<th>Requirements:</th>
<th>Approval from:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Vendor reps performing equipment demos using live animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Approval from:</td>
<td>Handle Animals?</td>
<td>Training</td>
</tr>
<tr>
<td>2. Vendors needing to access animal facilities to install or repair equipment in animal facilities (e.g. exterminators)</td>
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<td></td>
</tr>
<tr>
<td>3. Vendors bringing in potential customers to see equipment purchased and installed at Rutgers.</td>
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<td></td>
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<tr>
<td>a. Under a formal agreement at the time equipment was purchased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. a. Non-Rutgers students, interns, externs, volunteers working with faculty researchers or CMR (e.g. veterinary students, college students, vet tech students, high school students) who will have contact with animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Non-Rutgers students, interns, externs, volunteers who will not have contact with animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>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)</td>
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<td></td>
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<tr>
<td>6. Animal facility tours for non-Rutgers personnel</td>
<td></td>
<td></td>
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<tr>
<td>a. NJABR or University sponsored student groups</td>
<td></td>
<td></td>
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<tr>
<td>b. Potential contract customers who are considering doing animal work in Rutgers facilities</td>
<td></td>
<td></td>
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<tr>
<td>c. Media/legislator tours</td>
<td></td>
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<tr>
<td>7. Attendees at a workshop taught at Rutgers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. e.g. IACUC has procedures in place for Keck Center SCI workshops</td>
<td></td>
<td></td>
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<tr>
<td>8. Visiting scientists</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Human Medical and Dental consultants</td>
<td></td>
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</tr>
</tbody>
</table>

*Animal Care and Use orientation training is included as part of the course or workshop curriculum.
** The Rutgers IACUC will accept training completed at another institution if it is comparable to the Rutgers orientation program. Documentation must be submitted to the IACUC Administrator or CMR Director for review.
e. Visitor Flowchart

VI. References

a. VUMC policy on Minor in Laboratories: http://kc.vanderbilt.edu/mnlcore/Visitors.pdf
I. **Purpose** – The purpose of this document is to ensure that field studies involving animals at Rutgers University 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** - Rutgers University is committed to ensuring that all of its researchers conducting field studies are following the requisite regulations. Field studies are subject to a wide body of regulations on the international, federal, and state levels. Where the Animal Welfare Act (AWA) and the Public Health Service (PHS) Policy apply, the Institutional Animal Care and Use Committee (IACUC) is required to review all protocols involving vertebrate animals, 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 material 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.

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 research on wild animals and 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 timing 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 Rutgers University faculty and associated laboratory staff conducting field studies for the purposes of research and teaching.

**Principal Investigator (PI)** – PIs must be knowledgeable about animal welfare, relevant zoonotic diseases, associated safety issues, and any laws and regulations that apply. Where international regulations and policies apply, it is the responsibility of the PI to understand them, and to obtain any necessary permits/licenses prior to the onset of research. 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 is required for field research with animals and animal tissues that pose zoonotic disease risk.

In general, field study sites are exempt from inspection by the IACUC. 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.” Neither the Guide nor PHS Policy addresses the issue of inspecting field studies. However, 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”; any such recordings must follow the [Photography of Laboratory Animals at Rutgers](https://www.rutgers.edu/)
document.

IV. Definitions

a. **Animal** – Any vertebrate animal used for research, teaching, testing, or exhibition purposes.

b. **Field Study (non-exempt)** – Any study conducted on free-living wild animals in their natural habitat or any study involving animals not housed in the University animal facilities. Field studies may involve invasive procedures, and/or the animal’s behavior is materially altered. Procedures may include, but are not limited to, surgery (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.

c. **Exempt Field Study** – A study which does not involve animal manipulation and does not cause material alteration of the behavior of any animal. Generally, this type of study would involve behavioral observation, assessing population or impact to habitat, or collection of hair or feces from the environment (but not directly from the animal). Different funding agencies have different requirements, check with the funding source to determine the need for an IACUC-approved protocol if in doubt.
d. **AWA regulated species** — Includes dogs, cats, non-human primates, rabbits, guinea pigs, hamsters, marine mammals, and farm animals used for research as well as mice and rats not bred for research. The AWA requirements only apply to animals within the United States.

V. **Methods**

The information listed below must be considered and included in the protocol application if a field study is being planned. Deviations from the protocol must be documented immediately and reported to the IACUC within 5 days of reaching a location where communication can occur.

- **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. The IACUC reserves the right to consult with subject matter experts with relevant expertise.
- **Site Selection**: The investigator should explain how the chosen study location will maximize the opportunity for data collection and minimize disruption to the animals and their environment caused by the investigator. Information on supervision at the site should be included as well.
- **Methodology Employed**: The potential short- and long-term effects of procedures on individual animals should be described. Provide species-specific information as appropriate.
  - 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 make sure that the animals are not in distress and properly taken care of in terms of food, water, etc.
  - If animals are to be monitored individually, describe whether they will be identified by natural markings or artificially marked. If they are artificially marked, the protocol must describe the procedures associated with the marking.
  - Describe the possible impact of capture on subsequent behavior and survival of animals.
  - Describe sampling methods to be used following capture (e.g., blood collection, 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).
  - Describe measures taken to prevent injuries and alleviate pain and distress during capture, trapping, marking or sampling, and a contingency plan for adverse events that may occur in the process.
  - Describe whether individual animals are treated experimentally by surgery or drugs to alter their behavior or physiology. Any invasive surgery, such as organ removal or implanting of transmitters, must be done using aseptic technique.
  - Describe and justify any use and choice of anesthesia, including whether field conditions render certain agents too difficult to transport or use. Consideration should be given to drug availability at the field location and if there are potential barriers to import, transport or storage. Describe and justify any euthanasia.
  - Describe and justify procedures involving site manipulation such as the addition of a predator in well-justified cases. If fences are erected to limit movement of individuals or populations, the impact on other species should be considered.
  - If animals are to be transported during the study, describe the precautions that will be taken to secure the animals, prevent hypo- or hyperthermia, and reduce stress during transport. Another consideration is to employ practices to decrease the likelihood of capture myopathy. Capture myopathy is a disease complex associated with the capture or handling of any wild animal.
  - Describe the precautions taken by the researchers working with animals to protect themselves from possible zoonotic diseases or injury.
Describe any experiment that may cause potential distress, what outcomes are likely and whether the impact (or outcome) is within the range of ‘normal’. For example, a robotic stimulus used to simulate an actual lion for the measurement of normal responses of vigilance and flight, animals are minimally disturbed but the range of the outcomes is normal. In other words, are the behavioral responses likely to be extraordinary (troubling) or not?

Describe potential alternative methods that may result in the reduction, replacement, or refinement of those activities that may cause pain or distress. Describe why those alternatives are not appropriate for your research.

- The investigator must certify to the IACUC whether physiological or behavioral data collection methods are minimally invasive. When possible, minimally invasive procedures must be used.
- Euthanasia of wildlife in the field can raise unique and challenging issues that must be addressed by the investigator. The investigator should consult the most current version of the AVMA Guidelines for the Euthanasia on Animals, which includes considerations and techniques for euthanasia of wildlife.
- The investigator must assure the IACUC that all necessary federal and state permits have been or will be obtained before the research begins. In addition, if the research is being conducted in a foreign country, the investigator must assure the IACUC that all necessary local permits have been or will be obtained before the research begins. See Appendix A.
- The investigator must assure that all personnel working in the field will be properly trained and knowledgeable of the procedures as they are detailed in the protocol.

### a. Permitting Agencies

- **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.


- **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. For more information, go to [http://www.fws.gov/laws/lawsdigest/esact.html](http://www.fws.gov/laws/lawsdigest/esact.html)

- **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.

- **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/](http://www.nmfs.noaa.gov/pr/laws/mmpa/)

- **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

vii. **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

b. **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.

i. **Bureau of Land Management** (BLM): It has no specific requirements or permits for scientific research activities.

ii. **National Parks**: The National Park Service (NPS) has no specific regulation pertaining to scientific research. The NPS policy for research is found in its Administrative Guide, which 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).

iii. **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.

iv. **National Wildlife Refuges**: When a national wildlife refuge is created, it is considered closed to the public until it is expressly opened by its manager.

c. **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.

VI. **References**


d. OLAW FAQ #4 Program Review and Inspection of Facilities: Is the IACUC required to inspect field study sites?


f. Mpala Research Center: http://www.mpala.org/
I. **Purpose** - This policy establishes guidelines for daily observation of all animals maintained in Rutgers University animal facilities and investigator laboratories.

II. **Introduction** - The Public Health Service (PHS) and the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC, Intl) require that institutions comply with the standards in the *Guide for the Care and Use of Laboratory Animals*. The Institutional Animal Care and Use Committee (IACUC) must comply with the national standards and recommendations contained within 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 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*, p112

III. **Responsibilities** – This document applies to all research animals at Rutgers University, including satellite facilities and investigator laboratories.

IV. **Definitions** - none

V. **Methods** - All animals of every species (including fish, amphibians and reptiles) must be observed at least once every day of the year, including weekends and holidays. Room log sheets must be completed daily to document the observation. Observations are made by Animal Care Staff members in Comparative Medicine Resources (CMR) maintained animal facilities but in satellite facilities and investigator laboratories it will be done by the laboratory staff.

VI. **References**

I. Purpose – The purpose of this document is to provide animals users with guidance if they plan to house their animals on wire-bottom cages.

II. Introduction

According to the Guide (8th edition) -
“When given the choice, rodents prefer solid floors (with bedding) to grid or wire-mesh flooring”
- page 52

“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”
- page 51

“The following topics should be considered in the preparation of the protocol by the researcher and its review by the [Institutional Animal Care and Use Committee] IACUC:

- Nonstandard housing and husbandry requirements. Rutgers IACUCs interpret this as including wire-bottom caging.”
- pages 26-27

While AAALAC International does not provide an official “Position Statement”, the AAALAC Council 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.

AAALAC states:
- Every use of wire caging should be justified and approved by the IACUC.
- When the use of wire-bottom cages is approved, the institution needs to have a careful assessment program in place to monitor the health of the animals, particularly the feet of larger animals on longer studies.
- Institutions should be prepared with resting boards or other ways to provide animals that develop foot lesions relief from the wire and time to heal.
- Animal care and use professionals should continue to look for creative ways to enhance animal well-being by providing enrichment in wire cages. As with any enrichment technique, the research should not be negatively impacted by its presence.

III. Responsibilities – This document applies to all animal users at Rutgers University.

IV. Definitions

a. CMR – Comparative Medicine Resources
V. Methods

a. Use of Wire-Bottom Caging – Wire-bottom caging is considered an exception to the Guide and therefore requires strong scientific justification to the IACUC prior to its use.
   i. Animals must be housed on wire-bottom caging for the minimum duration necessary to achieve the experimental goals.
   ii. If possible, a solid resting surface should be provided for animals in each wire-bottom cage.
   iii. All animals housed on wire-bottom caging should have their feet inspected at least once a week if housed for greater than seven days. All observations should be recorded; these records may be reviewed at any time by IACUC members and/or CMR staff.
   iv. Suitable enrichment must be provided for animals housed on wire-bottom caging.

b. Metabolic Caging - Metabolic caging typically employs a wire-bottom floor construction to allow for easy collection of urine and feces; therefore the points outlined in section V.a of this document apply to metabolic caging.

VI. References

a. Guide for the Care and Use of Laboratory Animals, NRC Press, 2011

I. **Purpose** - This document provides instruction and guidance for providing environmental enrichment to laboratory species. The objective is to increase species-specific behavior while decreasing pathological maladaptive behaviors (stereotypy) by providing the animals with an enriched environment.

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.”

-the Guide, p52-53

“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.”

-the Guide, p51

III. **Responsibilities** – This document applies to all laboratory animal users at Rutgers University. At a minimum, all species must be provided with enrichment as specified in their species-specific husbandry SOP unless previously approved by the Institutional Animal Care and Use Committee (IACUC) as an exception in the protocol.

IV. **Definitions** - none

V. **Methods**

a. **Enrichment** – Each Rutgers campus has their own ‘default’ enrichment based on species. Examples of acceptable enrichment can include the following:

i. **Burrowing/Nesting Opportunities** - Rodents housed in solid bottom caging may have the following added to the cage to promote burrowing or nesting behaviors: paper rolls, paper towels, nesting sheets, Nestlets®, Enviro-dri®, foraging boxes, or additional bedding. In sterile rodent microisolation 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 nesting behavior.
ii. **Food Treats** - Food treats may be provided as part of the enrichment program. Suitable food treats for rodents include a variety of seeds or sugary treats. Food treats for large animals, depending on species, can include such items as fruit, vegetables, and/or sugary treats. In sterile rodent microisolation cages, any introduced food treats must be irradiated or sterilized in accordance with proper husbandry techniques.

iii. **Foraging Opportunities** – Food treats may be mixed with bedding to encourage foraging behavior.

iv. **Shelters** - PVC tubes, plastic shelves, or plastic/paper shelters (e.g. BioServ® huts and igloos or Shepherd Shacks®) may be placed in cages to allow animals to hide or escape from more aggressive cage mates.

v. **Gnawing Opportunities** - Nylabones®, chew sticks/blocks, plastic chains, cornhusks, cuttle bones, and Critter Cubes® promote gnawing behaviors.

vi. **Manipulanda** – Toys such as mirrors, balls, dumbbells, and hanging chains can be placed in animal enclosures to provide tactile enrichment opportunities.

vii. **Exercise Opportunities** - Running wheels, InnoWheel™ or Fast Trac for BioServ® igloo devices may be added to rodent cages where cage size and study constraints allow. Note that in some specific scenarios running wheels may be associated with stress/anxiety (such as use of running wheels in mouse models of anorexia, aka activity-based anorexia).

viii. **Examples of Species-Specific Enrichment**

1. **Rats**: social housing, burrowing or nesting opportunities, sheltering products, food treats, gnawing opportunities
2. **Mice**: social housing, burrowing or nesting opportunities, sheltering products, food treats, gnawing opportunities
3. **Guinea Pigs**: social housing, sheltering products, food treats, gnawing opportunities
4. **Swine**: social housing, manipulable toys, food treats, human interaction
5. **Rabbits**: social housing, manipulable toys, hay, elevated shelves, food treats, exercise opportunities
6. **Hamsters**: burrowing or nesting opportunities, sheltering products, food treats, gnawing opportunities
7. **Frogs**: social housing, sheltering products, ramps or platforms, floating items to hang onto, live prey
8. **Zebrafish**: social housing, imitation of natural substrate, artificial plants, shelters, sheltering products
9. **Birds**: social housing, perches, cuttle bones, baths, food treats, live prey

**Special Considerations** - Animals that show signs of being in psychological distress through behavior or appearance must be provided special attention. The Director of Comparative Medicine Resources (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.
Be aware that multiple enrichment items in a single cage (so called ‘superenrichment’), or inappropriate enrichment items may actually cause stress/anxiety.

b. Social Housing

Group housing must be practiced as the default for all social species. Single housing must be justified based on experimental requirements or veterinary related concerns about animal well-being. Social grouping may be suspended upon recommendation of a CMR veterinarian or Principal Investigator for health or study-related purposes. In these cases, it should be limited to the minimum period necessary. In the absence of other animals, an extra enrichment item must be offered to all rodents.

c. Exemptions to this Document – Note that in all cases of enrichment withholding or single housing of animals requires prior approval by the IACUC.

i. Enrichment – It has been shown that in some instances (such a neurological / behavioral studies) enrichment can alter the outcome of an experiment. In such cases enrichment can be witheld, if adequate scientific justification is presented to and approved by the IACUC prior to implementation.

ii. Social Housing (single housing of social species)

1. Animal Territorial Fighting - Some investigators maintain colonies of animals where fighting may occur if paired or grouped housed (e.g. breeder male mice). The intent is to have all investigators who breed animals include this exemption in their protocols, in addition to any other adequate justifications for single housing.

2. Food Uptake / Urine-Feces Collection – Animals for which food/water consumption or urinary/fecal output is being measured (such as metabolic studies) may have to be singly housed in order to collect accurate data.

3. Paralyzed Animals – Animals that experience paresis/paralysis may have to be singly housed to avoid injury from cage mates and being out-competed for food.

4. Post-Operative Recovery – Animals can be singly-housed in the immediate post-op period (<12hrs) as they are often susceptible to injury from cage mates until they fully recover from anesthesia. If animals need to be housed singly for longer than 12 hours, justification must be included in the protocol.

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).

Each time an animal is singly housed under these conditions, it will be reported to the Principal Investigator and the IACUC. If this happens more than once in a month, the investigator will be required to amend the protocol to include a justification for single housing.
VI. References


I. **Purpose** – The purpose of this document is to provide a way for laboratory animals to be transferred/adopted to a private owner as a companion animal.

II. **Introduction** – Rutgers University is committed to providing the highest level of animal welfare to laboratory species. In some cases, it is possible and desirable to adopt laboratory animals to individuals at the conclusion of a study or if there is a surplus of animals in order to avoid having to euthanize said animals.

III. **Responsibilities** – The Rutgers Institutional Animal Care and Use Committee (IACUC) is responsible for approving any animal adoption(s) with the assistance of the Comparative Medicine Resources (CMR) department.

Once the approval process is complete and the animal(s) have been removed from the Rutgers campus, any subsequent health checks and/or veterinary care is the sole responsibility of the new owner. Rutgers University assumes no obligation or responsibility for laboratory animals once the adoption process is complete.

All animals must be neutered before adoption unless pre-approved by a CMR veterinarian.

IV. **Definitions** - none

V. **Methods**

a. Adoption of animals may be considered with approval of a subcommittee of the IACUC. First contact for consideration should be to the CMR Director, who will present the justification to a subcommittee of the IACUC. Animals can only be adopted if they are not needed for further use by the University.

b. Animals that experienced major surgery (as defined by the Rutgers IACUC), have had health complications for any reason, have been exposed to any substance considered a (bio)hazard, or otherwise deemed unfit for adoption by a CMR veterinarian, are not eligible for adoption.

VI. **References** - none
I. **Purpose** - This document covers the procedures to be used by Rutgers University investigators and staff to sanitize restraint, enrichment, or other ancillary equipment used in animal studies conducted by investigators in laboratories (including satellite facilities) and maintained by research faculty and staff. This does not include equipment maintained by the Comparative Medicine Resources (CMR) department.

II. **Introduction** - Animal caging is normally cleaned and sanitized by animal care staff in accordance with relevant regulations (see section e.(iv) below). These regulations pertain as well to ancillary equipment that comes in contact with animals. This document provides assurance that such ancillary equipment is adequately sanitized and made safe for use in accordance with regulations when it is maintained by members of the research staff.

III. **Responsibilities** - Principal Investigators or designees are responsible for implementation and oversight of these procedures. Principal Investigators are required to keep a log sheet documenting dates of restrainer cleaning for all species covered under the Animal Welfare Act.

IV. **Definitions** - none

V. **Methods**

a. **Recommended Cleaning Agents**

i. **Clidox**
   1. 1:18 dilution
   2. Minimum contact time: 3 minutes at 20°C on pre-cleaned surfaces

ii. **MB-10**
   1. Prepare according to manufacturer recommendations
   2. Minimum contact time: 10 minutes

iii. **Quatricide**
   1. 1:5 dilution
   2. Minimum contact time: 10 minutes at 20°C on pre-cleaned surfaces

iv. **Strong Bleach Solution**
   1. ¼ cup of bleach to 1 gallon of cool water or 1 tablespoon of bleach to 1 quart of cool water (add the bleach to the water in either case).
   2. Minimum contact time: 2 minutes
v. Weak Bleach Solution
   1. 1 tablespoon bleach + 1 gallon of cool water
   2. Minimum contact time: 10 minutes

b. Restrainers and Enrichment Devices

   i. Restrainers or devices are to be cleaned at the end of the work day and between individual animal use.

   ii. Wash and rinse in a mechanical cage washer, or

   iii. Hand wash
       1. Wash used restrainer or device to remove all soil
       2. Soak or spray equipment with a suitable sanitizer (see list above, section V.a)
       3. Soak or let sit (for sprayed items) for at least the minimum contact time for the product used.
       4. Rinse with clean water, allow to dry.

c. Test Chambers

   i. Test chambers or devices are to be cleaned at the end of the work day and between individual animal use.

   ii. Remove all loose bedding, feces and other materials from chamber.

   iii. Wipe down or spray all surfaces with one of the recommended cleaning agents and allow to sit for the minimum contact time.

   iv. Wipe down surfaces with clean water.

d. Stereotaxic and other Surgical, Technical, or Experimental Equipment

   i. Stereotaxic and other surgical, technical or experimental equipment or devices are to be cleaned at the end of the work day and between individual animal use.

   ii. Wipe all surfaces with disinfectant e.g. Quatricide, or 70% ethyl alcohol.

   iii. Note – Surgical instruments must be sterilized before use and maintained using aseptic technique (see Rodent Survival Surgery and Non-Rodent Surgery/USDA Species documents for further information).

e. Notes

   i. 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.

   ii. If residual odors from the cleaning chemicals might affect study animals, or if chemicals may damage equipment, a special request for exemption from sanitizing equipment must be obtained.
from the Institutional Animal Care and Use Committee (IACUC) as part of an approved animal use protocol.

iii. Quaternary ammonium compounds should be used with caution around breeding animals, as studies have demonstrated decreased fertility in rodents exposed to these chemicals.

iv. The Animal Welfare Act (9 CFR Ch.1 §3.11(b) (1)) 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].

The Animal Welfare Act (9 CFR Ch.1 §3.11(b) (3)) requires 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.

See also: *Guide for the Care and Use of Laboratory Animals* sanitation requirements (8th ed, pages 69-72).

VI. References


I. **Purpose** - This document addresses the designated member review (DMR) procedures to be used by Rutgers University institutional animal care and use committees (IACUCs).

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 Principal Investigators (PIs) are responsible for complying with policies and procedures outlined in this document.

IV. **Definitions** - none

V. **Methods**

a. **Designated Member Review in Lieu of Full Committee Review (DMR in lieu of FCR)**

i. **Categories of protocols routed to DMR in lieu of FCR**

1. USDA:
   a. New and De Novo protocols at stress levels “C”
   b. All Continuing Reviews, regardless of stress level
   c. Amendments that do not raise the stress level to “E”

2. Non-USDA:
   a. New and De Novo protocols at stress levels “C” and “D”
   b. Amendments that do not raise the stress level to “E”

3. No other categories of protocols may undergo DMR in lieu of FCR

4. 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.

ii. **Procedure for DMR in lieu of FCR**

1. IACUC members are notified of all protocols routed to DMR in lieu of FCR at least once daily and have access to all protocols undergoing DMR.

2. IACUC members are asked to notify the IACUC Office should they object to DMR in lieu of FCR within two University operating days, starting the day after receipt of the submission.

3. 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.
4. If the PI has not responded to questions from the IACUC within 60 days, the submission may be administratively withdrawn.

b. Designated Member Review Subsequent to Full Committee Review (DMR following FCR)

i. Categories of protocols that may undergo DMR subsequent to FCR
   1. Any type or stress level protocol may undergo DMR following FCR

ii. Procedure for DMR subsequent to FCR
   1. 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.
   2. 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).
   3. If the PI has not responded to questions from the IACUC within 60 days, the submission may be administratively withdrawn.

VI. References

I. Purpose: The purpose of this document is to provide guidelines on identification of live rodents. This policy only applies to laboratory mice (of the genus *Mus*) and rats (of the genus *Rattus*). 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 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, relying on cage card level identification alone may result in misidentification of individual animals.

IV. Definitions: none

V. 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 (short term use):** For short term identification, non-toxic, indelible markers can be used to color the fur or tail of an animal. Because the coloring fades quickly, such identification should only be relied on for short periods of time (usually 3-4 days) unless the color is reapplied. There are no age restrictions for color staining an animal’s skin or fur.

v. **Toe clipping:** Toe clipping is allowed in rats up to 7 days of age and mice up to 17 days of age. If animals are over this age limit, toe clipping must be scientifically justified in an approved protocol, and used as both a method of identification and for tissue collection. The best age in mice is between 5-17 days as it appears to have fewer adverse effects on behavior and well-being until this age.

Directions:

i. Use small, sharp, sterile scissors (such as ocular microsurgical scissors).

j. Cut only one toe from each paw. Therefore, a maximum of 4 toes may be cut. If the mouse is between 8-17 days of age, only 2 total toes may be cut.

k. Remove only the distal phalanx, and remove the entire nail bed to avoid regrowth.

l. Do not cut the hallux (aka dew claw, thumb, little toe of the forepaw) as this may decrease the rodent’s grasping ability.

m. Start with the toes of the hind feet as they are less sensitive than the front toes.

n. Procedure:
   
   i. Wipe down the skin of the foot and digits with alcohol.
   
   ii. Gently restrain the rodent.
   
   iii. Extend the leg and remove distal phalanx.
   
   iv. 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 adult mice can be implanted with commercially available microchips or transponders that can be scanned using an electronic handheld 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 (≤16ga), more than momentary pain is associated with their use. Therefore, **all procedures involving implantation 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. 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:

<table>
<thead>
<tr>
<th>Identification Method</th>
<th>Age Restriction</th>
<th>Anesthesia Necessary</th>
<th>Analgesia Necessary</th>
<th>IACUC Approval Necessary</th>
<th>Additional Information</th>
</tr>
</thead>
</table>
| Ear notch/punch/clip  | None            | No                    | No                  | No                       | • Punch or scissors should 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                  | Necessary if performed under general anesthesia | • Site should be disinfected prior to use. |
| Colored stain         | None            | No                    | No                  | No                       | • Site may need to be marked repeatedly every 3-4 days. |
| Toe clip              | **Mice:** Up to 17 days of age (recommend 5-17 days of age)  
                                                                              **Rats:** Up to 7 days of age | Necessary after 17 days of age in mice or 7 days of age in rats. Toe clipping after these ages must be justified in an IACUC protocol. | Necessary after 17 days of age in mice or 7 days of age in rats. Toe clipping after these ages must be justified in an IACUC protocol. | Yes  
                                                                              • Instruments should 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).  
                                                                              • If animal is between 8-17 days, only 2 total toes may be cut.  
                                                                              • Hemostasis must be achieved.  
                                                                              • May impair grip strength.  
                                                                              • Tissue can also be used for genotyping. |
| Subcutaneous transponder | Recommended to perform around or after weaning age | General and local anesthesia necessary | Local anesthesia at time of implantation necessary | Yes                       | • Implantation considered minor surgery by IACUC.  
                                                                              • Site should be disinfected with 3 alternating applications of surgical scrub and alcohol before placement.  
                                                                              • May not be compatible with protocols involving advanced imaging (MRI, CT).  
                                                                              • May lead to tissue response or neoplasia with prolonged placement. |
viii. Appendix

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).
VI. References


I. **Purpose** - The purpose of this document is to allow for more efficient amending of IACUC protocols, where changes conform to the following guidelines and do not negatively impact animal welfare.

II. **Introduction** - Guidance from OLAW provides the IACUC with some discretion to handle specific protocol changes administratively, in accordance with IACUC approved policies through consultation with a veterinarian authorized by the IACUC. In certain circumstances investigators will desire to amend details of a protocol which do not require a full committee review. Certain changes only require verification of a Rutgers veterinarian; this document details which changes qualify for VVC.

III. **Responsibilities** - IACUC administrators will route amendments considered for veterinary verification and consultation directly to one of the Rutgers veterinarians in lieu of either FCR or DMR.

IV. **Definitions**
   a. DMR – Designated Member Review
   b. FCR – Full Committee Review
   c. IACUC – Institutional Animal Care and Use Committee
   d. VVC – Veterinary Verification and Consultation

V. **Methods**
   a. The following changes **must be approved by the IACUC**, either through FCR or DMR, in accordance with regulatory and Rutgers policies:
      i. 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 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 to those already approved in the protocol
      ix. Addition of a non-pharmaceutical drug – this includes drugs that may have been approved in a pharmaceutical formulation
      x. More than 10% increase in the total number of animals required
      xi. Any increase in animal numbers used in the Category E component(s) of the protocol
      xii. Change in food or water modification intended to affect experimental outcome(s)
      xiii. Change in post-procedural monitoring and restraining methods

   b. The following changes **may be reviewed by VVC**:
      i. Changes in drugs used for anesthesia, analgesia, sedation (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%) due to unforeseen circumstances or increased production of offspring in a breeding colony

iv. Addition of a new compound in the same class of compounds as those already approved in the protocol

v. Change in dosing schedule, change in procedure time points, and/or extension of the experiment time

vi. Addition of a new genotype, strain, or breed of animal provided it does not change the objectives of the study or where new procedures will be added

vii. Change in an existing procedure resulting in less pain, distress, or degree of invasiveness

c. The veterinarian may refer the request to the IACUC for review for any reason and must refer any request that does not meet the parameters of the IACUC policies.

VI. References - https://olaw.nih.gov/guidance/significant-changes.htm