"Anesthesia, Analgesia, & Euthanasia"

-Rodent Seminar Series Documents-

JHU ACUC Website: https://web.jhu.edu/animalcare/

New / 3rd Year Renewal Protocol Form ***(CURRENTLY UNDER REVISIONS, NEW FORM ONLINE SOON)***

RAR Website: https://researchanimalresources.jhu.edu/

ACUC Guidelines: https://web.jhu.edu/animalcare/policies/index.html

1. Use of Anesthetic Gases: General Guidelines/Vaporizer Calibration
2. Use of Anesthetic Gases: "Drop method"
3. Use of "Non-Pharmaceutical Grade" Substances in Laboratory Animals
4. Record-keeping, Storage, and Disposal of Drugs Scheduled under the Controlled Substances Act (CSA)
5. Rodent Drug Formulary: Quick Reference Guide
6. Use of Neuromuscular Blocking Agents
7. Approval of Use of Sustained Release Formulations of Buprenorphine HCL in Currently Approved Protocols
8. Guidelines on Analgesia for Rodent Surgeries
9. Euthanasia of Mice and Rats Using Carbon Dioxide

Other Useful Information:

10. *Guide for the Care and Use of Laboratory Animals 8th Edition (2011)*
    https://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth
11. *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*
    https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf
12. Fidelis – Ethiqa XR (Buprenorphine extended-release): https://ethiqaxr.com/

Johns Hopkins University
Animal Care and Use Committee

Use of Anesthetic Gases: General Guidelines/Vaporizer Calibration[1]

PURPOSE: The Animal Care and Use Committee (ACUC) has developed the following guidelines to control risk of exposure to waste anesthetic gases in the workplace.

A. INTRODUCTION

Inhalant anesthetic gases (e.g. isoflurane, halothane, sevoflurane, desmoflurane etc.) are halogenated gases that are commonly used in animal research. Halogenated anesthetics are typically clear, colorless, highly volatile liquids at normal temperature and pressure. Exposure to these substances occurs when vapors escape into the work environment during the anesthetic administration process.

Waste anesthetic gases possess very poor warning properties so odor is not an adequate indication of overexposure. Long-term exposure to waste anesthetic gases has been linked to genetic mutations, cancers, spontaneous abortions, hepatic and renal disease and psychomotor changes in humans. Health hazard information is available from the Toxnet database at http://toxnet.nlm.nih.gov/.

B. PROCEDURES TO REDUCE EXPOSURE

Equipment and system maintenance for anesthesia machines and vaporizers

1. All anesthetic vaporizers must undergo calibration verification by a professional service technician as recommended by the manufacturer and be serviced if necessary.
2. If no such recommendation exists, the following schedules apply:
   Halothane vaporizers- Calibration verification must be performed *annually* because halothane's properties lead to increased clogging of internal vaporizer components.
   Isoflurane vaporizers- Calibration verification must be performed at least *every 3 years*. If the machine is subject to extensive use (e.g., > 500 hrs/year) or is frequently moved to different locations, then verification must be performed annually.
3. A copy of the manufacturer's guidelines for calibration verification must be available in the laboratory to assist with oversight by the ACUC of proper maintenance of anesthetic equipment.
4. Documentation of equipment validation must be affixed to each anesthesia machine or vaporizer that is in service.

Environmental controls

1. Work in a well-ventilated area such as a laboratory or operating room.
2. Whenever possible, handle liquid anesthetic agents in a certified chemical fume hood, hard-ducted biosafety cabinet, downdraft table, or use another suitable local exhaust system.

Protective garb such as gloves, lab coat, and eye protection (face shield or goggles) should be worn when dispensing anesthetic agents.

3. Use a reliable gas scavenging system to collect, remove and dispose of waste anesthetic gases. Scavenging options include:
   a. Dedicated exhaust system: A dedicated exhaust system such as an active vacuum waste gas line or an "elephant trunk" exhaust system is the preferred method to remove waste gases from the work environment.
   b. Non-circulation ventilation systems: These discharge waste gases through an exhaust vent or grill (e.g., hard-ducted biosafety cabinet or downdraft table).
   c. Chemical fume hood: The anesthetic can be delivered to the animal while it is inside the fume hood or an exhaust gas line from the anesthesia machine can be vented inside the hood.
   d. Adsorption devices: Charcoal canisters such as F-Air or Enviro-Pure can be used to absorb halogenated waste gases. These canisters must be properly placed so that the vent holes on the bottom of the canister are not obstructed. Usage must be documented and accompanied by the method used to determine canister life as supplied by the manufacturer. For F-Air canisters this involves weighing the canister before and after use and discarding the canister when there is a 50 g increase from the initial weight.

C. TRAINING AND SOP REQUIREMENTS

PI's are responsible for training their staff who work with anesthetic gases before use.

D. ADDITIONAL USEFUL INFORMATION

1. Fish, R., Danneman, P., Brown, M., Karas, A., (Eds.). *Anesthesia and Analgesia in Laboratory Animals*, New York: Academic Press (2008).
2. Federal OSHA Fact Sheet Number 91-38 (Waste Anesthetic Gases)
http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=FACT_SHEETS&p_id=128
3. OSHA Guidance Document – ANESTHETIC GASES: Guidelines for Workplace Exposureshttp://www.osha.gov/dts/osta/anestheticgases/index.html
4. For help with anesthesia delivery systems and techniques contact a Research Animal Resources (RAR) veterinarian at 410-955-3273.

[1] Approved by the Animal Care and Use Committee: October 15, 2009; *reviewed 1/5/15*

Johns Hopkins University
Animal Care and Use Committee

Use of Anesthetic Gases: "Drop method"[1]

PURPOSE: When performing short-term procedures with mice and rats, the "Drop Method" may be preferred for use of inhalational anesthesia as it does not require use of a vaporizer and flow meter.

OCCUPATIONAL SAFETY: To reduce occupational health risk that exists from chronic exposure to anesthetic gases, it is recommended that these procedures, as well as pouring of liquid anesthetic agents, be performed in a chemical fume hood, biosafety cabinet, downdraft table, or by use of other local exhaust methods, whenever possible. If none of this equipment is available and you wish to perform the procedure on an open bench top, the Health, Safety and Environment (HSE) office must be contacted and exposure levels measured and approved. After HSE grants approval, the ACUC office must be informed of the location so that the exception can be documented for inspection purposes. Regardless of where the procedure is performed, appropriate protective garb (gloves, lab coat, and eye protection such as face shield or goggles) should be used when dispensing liquid anesthetic agents. For information on health risks associated with gas anesthetic agents go to http://toxnet.nlm.nih.gov/.

METHOD: The "Drop Method" consists of permitting anesthesia liquid (e.g., halothane, isoflurane) to vaporize in close enough proximity to the animal that it becomes anesthetized. This can be accomplished by exposing the animal to the vapor in a chamber or by exposing the animal to the vapor emanating from a tube placed near its nose.

Chamber: For brief procedures, a chamber (one that permits viewing the animal) that is large enough to accommodate the animal is used to anesthetize the animal. A small amount of anesthesia liquid is placed in either a reservoir or on an absorbable material, such as gauze. The reservoir, or material, must be positioned so the animal cannot come in direct contact with the anesthesia liquid.

Two methods are described below for determining the appropriate amount of a gas anesthetic to use in the chamber.

Diluted Gas Anesthesia (*PREFERRED METHOD*)
Diluted gas anesthesia is the preferred method of delivery because it minimizes lethal accumulations of anesthetic in the vapor phase and provides a wider margin of safety. A mixture of 20% v/v liquid anesthetic in propylene glycol is recommended for mice, and 30% v/v liquid anesthetic in propylene glycol is recommended for rats. A general guideline for the amount of diluted mixture is ~1.0 cc for every 500 cc volume of the chamber.

Undiluted Gas Anesthesia

When using undiluted gas anesthesia, it is **extremely** important to use the appropriate amount to prevent over-anesthetizing the animal. The amount of anesthesia liquid to use should be determined based on the volume of the chamber. The table below provides a guide to the percentage of vaporization that can be achieved in relation to the ml of liquid used per liter volume of the chamber. Anesthesia usually will be adequate at 1.0 – 1.5%.

ml per liter volume of container	Equals %
0.05	1
0.10	2
0.15	3
0.20	4

Example 1: A chamber that is 10 cm wide by 10 cm long by 10 cm high = 1000 cm^3 or a volume of 1.0 liter. Thus, for 1% vaporization, 0.05 ml of liquid anesthetic should be used.

Example 2: A chamber that is 8 cm wide by 10 cm long by 7 cm high = 560 cm^3 or a volume of 0.560 liters (1000 cm^3=1 liter). Thus, for 1% vaporization, 0.028 ml liquid anesthetic should be used.

Once the anesthetic has been loaded into the chamber, the animal is placed inside and monitored for unconsciousness. A deep plane of anesthesia is indicated by lack of a righting reflex when the chamber is tipped slightly. Once the animal is anesthetized, it can be removed from the chamber and checked to ensure an adequate depth of anesthesia before beginning the procedure. An effective method to evaluate whether you can proceed is to apply a toe pinch. If the animal responds to the toe pinch it is not adequately anesthetized and should be returned briefly to the chamber. When exposed to the air, the animal will wake up quickly so the procedure must be brief. The chamber must be sanitized between uses to prevent cross-contamination among animals.

Tube: For a slightly longer period of anesthesia, a tube (e.g., 15 ml conical tube for mice or a 50 ml conical tube for rats) can be loaded with gauze containing several drops of anesthesia liquid (slightly more if using the diluted form). The animal should be restrained and the nose held to the tube until the anesthesia takes effect. Alternatively, the animal can be anesthetized in the chamber and, after reaching the desired depth of anesthesia, the end of the tube can be placed over the nose of the animal to maintain anesthesia. It is important to assure that the gauze itself not come in contact with the animal. Care must be taken not to over-anesthetize, and thereby euthanize, the animal. By moving the tube closer or farther from the animal's nose, the level of anesthesia can be controlled. This method requires continuous monitoring of the animal.

Training in these methods can be arranged by contacting the ACUC office at acuc@jhmi.edu

[1] Approved by the JHU Animal Care and Use Committee on: October 15, 2009
minor revisions on January 28, 2010, January 31, 2018

Johns Hopkins University
Animal Care and Use Committee

Use of "Non-Pharmaceutical Grade" Substances in Laboratory Animals

PURPOSE:
This document describes requirements regarding the use of non-pharmaceutical-grade chemicals or compounds in laboratory animals at Johns Hopkins University to remain in compliance with positions held by the Office of Laboratory Animal Welfare (NIH-OLAW), the United States Department of Agriculture -Animal Plant Health Inspection Service (USDA-APHIS) and the *The Guide for the Care and Use of Laboratory Animals* (8th ed., 2011)

BACKGROUND:
Guidance from the National Institutes of Health (NIH) Office of Laboratory Animal Welfare (OLAW) requires that preference be given to "pharmaceutical grade" chemicals and other substances when selecting compounds for use in laboratory animals.[1] An OLAW policy statement[2] has defined "pharmaceutical grade" compounds as those that have been approved for human or veterinary clinical use by the Food and Drug Administration or those for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary, or British Pharmacopeia. Just as the delivery of drugs and other substances to laboratory animals must be approved by the ACUC, the choice to use non-pharmaceutical grade compounds must be approved as well. Fortunately, OLAW has indicated that the ACUC may employ "a variety of administrative methods to review and approve" the use of non-pharmaceutical grade substances, including the establishment of "acceptable scientific criteria within the institution, rather than on a case-by-case basis."[2] The purpose of this document is to accomplish this objective for JHU and to provide information about the policy to the JHU research community.

I. Drugs used for a clinical purpose in laboratory animals:

Consistent with OLAW requirements, the JHU ACUC expects that drugs that meet the OLAW definition of pharmaceutical grade compounds will be used for sedation/restraint, anesthesia, analgesia, euthanasia, and other clinical purposes in laboratory animals. This policy for use of pharmaceutical grade compounds for "medications" is also consistent with the policies of the

[1] Page 31, *Guide for the Care and Use of Laboratory Animals* (8th edition), published 2011. Adopted as an extension of PHS Policy by OLAW December 1, 2011.

[2]Policy statement on Non-pharmaceutical grade substances issued 12/1/2011 in response to public comment period ending 5/24/2011; revised 5/29/2012 in response to the public comment period ending 2/3/2012. http://grants.nih.gov/grants/olaw/positionstatement_guide.htm

United States Department of Agriculture's Animal and Plant Health Inspection Service for enforcing the Animal Welfare Act Regulations.[3]

If a preferred compound is not available as a clinical use formulation, a non-pharmaceutical grade formulation may be used if approved by the ACUC. This applies as well to compounds that typically are available in a pharmaceutical grade preparation but become unavailable for some period of time.

Although OLAW[1,2] does not consider "cost savings alone" an adequate justification for the use of non-pharmaceutical-grade compounds in animals, it has recognized that from time to time some drugs may be in such short supply, with an accompanying large escalation in cost, that cost may become a relevant variable for approval of a non-pharmaceutical grade compound.[4]

II. Drugs used as research tools or the subject of investigation:

The JHU ACUC recognizes that compounds that fall into the non-pharmaceutical grade category defined above are essential to basic research in many scientific fields, particularly in the bio-medical research that comprises the vast majority of the animal research protocols handled by the ACUC.

The JHU ACUC expects that investigators will consider relevant animal welfare as well as scientific issues in choosing the types and sources of substances to administer to laboratory animals for research purposes. Some of the circumstances that compel use of a non-pharmaceutical grade compound for research include the following:

- The compound is not available in a clinical use formulation.
- The compound is supplied to the investigator through an NIH Drug Supply Program.
- The compound is supplied to the investigator through a JHU-negotiated Material Transfer Agreement with a pharmaceutical or biotechnology firm.
- The investigator has a collaborative research relationship with an academic medicinal chemist to carry out a pharmacological characterization of novel compounds with potential for therapeutic use.

The JHU ACUC also recognizes that when a clinical use formulation has been marketed for a particular compound, it may not be useful for a research purpose due to one or more of the following variables:

- The need to manipulate concentration while holding volume constant. (Adding drug powder or diluting the pharmaceutical grade formulation would obviate its advantage.)
- Lack of the appropriate vehicle control.
- Formulation is inappropriate for the planned route of administration.
- Presence of preservatives or other undesirable components in the formulation.
- The need to hold formulation constant for comparison to previous studies.

As stated above, OLAW does not consider cost savings alone an adequate justification for the use of non-pharmaceutical-grade compounds in animals.[1,2] They have, however, recognized that from

---

[3] Policy 3, Veterinary Care (www.aphis.usda.gov/animal_welfare/policy.php?policy=3). Applies to species covered by the Animal Welfare Act (generally, mammals other than rats and mice that have been bred for use in research; birds not bred for research also are included).

[4] Statement by Susan Silk, M.S., Director of the Division of Policy and Education, OLAW: "AAALAC and OLAW.Implementation and Recommendations Regarding the New *Guide*. . . ." (Scientists Center for Animal Welfare IACUC Training Workshop, Baltimore, MD, April 30, 2012) in relation to the virtual unavailability of pentobarbital sodium except at a cost of $1000 for 50 ml of 50 mg/ml solution from very limited sources.

time to time some drugs may be in such short supply that cost may become a relevant variable.[4] Similarly, the JHU ACUC recognizes that due to a continuing need for a particular compound in a research, the pharmaceutical grade formulation may be so expensive as to preclude the investigator from using it on a continuing basis with the research resources available.

Expectations of the JHU ACUC for preparation and use of non-pharmaceutical grade compounds:

The ACUC expects researchers to have the expertise and professional judgment to determine the most appropriate formulation and route of administration for their research. If he/she determines that a non-pharmaceutical grade substance is needed, the ACUC expects that the researcher will obtain the highest quality/purity and will have the expertise, technical information, and laboratory resources for preparing a formulation that is most suitable for the planned route of administration.

Regardless of whether a compound is pharmaceutical or non-pharmaceutical grade, pharmacokinetics may be a variable relevant to choosing one route of administration over another for either clinical or research purposes.

The following variables may be relevant to consider, depending on route of administration, when formulating non-pharmaceutical grade substances for use in laboratory animals: sterility, acid-base balance, pyrogenicity, osmolality, and compatibility of components.[1,2]

As in any pharmacology study, researchers are expected to anticipate possible side effects and/or adverse reactions that may be relevant to normal functioning of the animal and state these, along with anticipated duration and/or need for treatment, in the protocol.

The JHU ACUC expects that the duration of storage and use of a non-pharmaceutical grade formulation will be compatible with the duration for which the formulation will remain potent, as per technical information available. Methods for preparing and storing formulations must prevent contamination that could adversely affect animal welfare or the interpretation of data. Formulations must be labeled with the name of the compound and the concentration as well as the date of preparation and planned date of disposal.

Johns Hopkins University
Animal Care and Use Committee

Record-keeping, Storage, and Disposal of Drugs Scheduled under the Controlled Substances Act (CSA)[1]

PURPOSE: This document provides guidance based on CSA regulations for the appropriate record-keeping, storage, and disposal of controlled substances for JHU investigators conducting research with animals.[2]

BACKGROUND: Registration to obtain and use drugs that have been legally scheduled under the CSA Regulations is issued to individuals by the DEA. Registration with the Maryland Department of Health and Mental Hygiene is required prior to DEA's approval of federal registration. Although the DEA rarely audits preclinical researcher records, Public Health Service Policy includes the requirement for proper storage and record-keeping of controlled substances used for "human and veterinary drugs and treatments,"[3] and AAALAC International covers this issue in its triennial site visits. Thus the JHU ACUC covers this issue in its semi-annual inspections.

Record keeping[4]: Record of receipt and use of a controlled substance needs to be maintained for each lot received. Below are the basic requirements. Other information can be included as desired by the investigator.

--Name of the drug
--Source
--Date received
--Manufacturer's lot number (on the container)
--Amount received (such that total amount of drug is shown or could be derived, such as, "10 g" if powder, or "10 2-ml vials, 5 mg/ml" if liquid)

One must be able to match drug containers on hand with the record, which can be done by use of the lot number. Labelling bottles or boxes of vials with the date of receipt can be helpful. If multiple bottles or boxes of vials from the same lot are received on the same day, then putting a code (e.g., A, B, or C) on the bottle or the box of vials can be helpful to track use of that drug lot.

As the drug is used, the following information needs to be recorded (a tabular/columnar format works best):

--Date of removal
--Number of units removed (e.g., 50 mg, if powder; or Bottle A, 1 ml).
--Amount remaining (i.e., a running total of amount remaining in a separate column)
--Name or initials of the person who removed that amount of drug

---

[1] Approved by JHU Animal Care and Use Committee: September 19, 2002. Revised April 20, 2006. Revised October 20, 2011. Revised November 19, 2015.

[2] The record-keeping requirements for animal research apply whether a the DEA registration is that for a researcher or as a "coincident activity" under a practitioner registration [21CFR1301.13(e)].

[3] *Guide for the Care and Use of Laboratory Animals*, 8th Ed., National Research Council, 2011; p. 115.

[4] This guidance has been derived from 21CFR1304.22. "Records for manufacturers, distributors, dispensers, researchers, importers and exporters."

--Purpose (this will vary across types of drugs and species. For some drugs, it may be sufficient to have a heading on the record page that states the purpose, such as, "Preparing solutions for anesthesia in mouse surgeries," "Sedation of monkeys for physical examinations").

If the drug is used to make a solution (e.g., from powder or by diluting with saline or combining two or more solutions) that will be maintained for multiple uses over some period of time, keep a separate record of use of that "working solution."

Records must be kept secure and readily retrievable for inspection, but there is no specific requirement on exactly where they must be kept. The packing slip for the shipment should be retrievable also, but it does not need to be kept in the same location as the record of use. Record of use and the packing slip for each lot are to be retained for 2 years.

Secure Storage: The stored controlled substances "shall be accessible only to an absolute minimum number of specifically authorized employees" (21CFR1301.72). The registrant determines who those individuals are to be. There are no paperwork requirements on continuing specification of those individuals once registration is approved. The approved registration application will have specified the means by which the license holder will assure secure storage of controlled substances. .

Security requirements vary under the CSA depending on the particular schedule of a compound (requirements are highest for Schedule I and II compounds), quantity of controlled substance; the location (e.g. laboratory vs. clinic), adequacy of key control and/or combination locks, extent of unsupervised public access, and availability of security personnel. Thus research laboratories at Johns Hopkins generally already are considered relatively secure given that they are not in areas frequented by the general public and have restricted access to employees per se. For example, the "double lock" criterion can be considered satisfied when there is (1) restricted access to the room, floor, or building and (2) restricted access to the controlled substance within the room.

Expired drugs still must be securely stored. Although PHS Policy and Animal Welfare Act interpretation do not permit use of expired drugs for clinical purposes, expired controlled substances are considered subject to abuse. They remain the responsibility of the registrant.

Disposal: In September, 2014, the DEA issued a final rule regarding the disposal of controlled substances that has simplified requirements for animal researchers [21 CFR 1317.10(b) "non-practitioner inventory"; 1317.90]. The basic consideration in drug disposal is prevention of diversion. One methodology is use of a Reverse Distributor, which is a company authorized to receive controlled substances and to destroy them. This is a method commonly used by pharmacies and clinical settings, but the cost is typically prohibitive for basic researchers.

The new regulations do not require a particular method of destruction so long as the substances are rendered "non-retrievable." This is defined as rendering the controlled substance "unavailable and unusable for all practical purposes." Although flushing controlled substances down a drain does make the drug non-retrievable, this practice no longer is considered consistent with the Clean Water Act. An example of a reasonable method of rendering a solution non-retrievable is in animal waste or squirted onto a paper towel that goes into a biohazard bag.

Destruction of small amounts of unused solutions (e.g., left in a vial, tube, or syringe) should be recorded in the record of drug use. Destruction of larger amounts (e.g., expired unopened vials or bottles of solution) is to be recorded on DEA Form 41, available on the DEA websitee with instructions.

Rodent Drug Formulary: Quick Reference Guide

(https://web.jhu.edu/animalcare/index.html)

MICE

Inhalant Anesthesia

Agent(s)	Dose	Comments/Reference(s)
Isoflurane	3-5% Induction 1-3% Maintenance	Administer via precision vaporizer and compressed oxygen or drop method

Injectable Anesthesia

Agent(s)	Dose	Comments
Ketamine Xylazine	80-100 mg/kg IP 5-10 mg/kg IP	Surgical anesthesia
Ketamine Acepromazine	100 mg/kg IP 5 mg/kg IP	Immobilization/anesthesia
Ketamine Midazolam	100 mg/kg IP 5 mg/kg IP	Immobilization/anesthesia
Ketamine Xylazine Acepromazine	80-100 mg/kg IP 10-20 mg/kg IP 2-3 mg/kg IP	Surgical anesthesia
Pentobarbital	40-60 mg/kg IP	Considerable dose variation by strain, gender, genetic modifications etc. Starting at low end of dose range is advisable. Note: Euthanasia dose is 90-100 mg/kg or greater
Tribromoethanol (Avertin)	200-500 mg/kg IP	Non-pharmaceutical grade; special preparation and storage required; Adverse effects likely with repeat dosing

Analgesia

Agent(s)	Dose	Comments
OPIOID		
Buprenorphine	0.05 - 0.1 mg/kg SC, IP q 8-12 hr	DEA required; Preferred analgesic for rodents
Buprenorphine SR™ Lab Sustained release buprenorphine	0.5-1.0 mg/kg SC q 48 hr *Mouse dose rage	Manufacturer: ZooPharm Note: Rat and mouse dose ranges are different. No longer requires refrigeration. **Prescription from RAR veterinarian required. Obtain forms from:** http://web.jhu.edu/animalcare/Instructions%20for%20ordering%20sustained%20release%20buprenorphine
NSAID		
Carprofen (Rimadyl ®)	4-5 mg/kg SC q 24 hr	
Meloxicam (Metacam ®)	1-5 mg/kg SC q 24 hr	

Local Block Analgesics

Agent(s)	Dose	Comments/Reference(s)
Lidocaine (1-2%)	Local infusion; do not exceed 7mg/kg	Onset: 5-10 min, Duration: 0.5-1 hr Several methods of administration (field block, infiltrative block etc.).
Bupivacaine (0.5% Marcaine)	Local infusion; do not exceed 8 mg/kg	Onset: 15-30 min, Duration: 4-8 hr Several methods of administration (field block, infiltrative block etc.).

RATS

Inhalant Anesthesia

Agent(s)	Dose	Comments
Isoflurane	3-5% Induction 1-3% Maintenance	Administer via precision vaporizer and compressed oxygen or drop method

Injectable Anesthesia

Agent(s)	Dose	Comments
Ketamine Xylazine	75-100 mg/kg IP 5-10 mg/kg IP	Provides a good surgical plane of anesthesia for most procedures
Ketamine Acepromazine	75 mg/kg IP 1- 2.5 mg/kg IP	Best used for prolonged restraint or minor surgical procedures
Ketamine Xylazine Acepromazine	40 mg/kg IP 5 mg/kg IP 1 mg/kg IP	Provides a good surgical plane of anesthesia for most procedures
Ketamine Midazolam	75-100 mg/kg IP 4-5 mg/kg IP	Best used for prolonged restraint or minor surgical procedures
Ketamine Dexmedetomidine (Dexdomitor ®)	75-100 mg/kg IP 0.15 mg/kg IP	Provides a good surgical plane of anesthesia for most procedures
Pentobarbital	40-50 mg/kg IP	May provide a surgical plane of anesthesia however there is a wide range of dose variability and often a narrow safety margin; caution should be used to avoid overdoses

Analgesia

Agent(s)	Dose	Comments
OPIOID		
Buprenorphine	0.01-0.05 mg/kg SC, IP q 6-12 hr	DEA required

Buprenorphine SR™ Lab Sustained release buprenorphine	1.0 -1.2 mg/kg SC q 72 hr *Rat dose range	Manufacturer: ZooPharm Note: Rat and mouse dose ranges are different. No longer requires refrigeration. **Prescription from RAR veterinarian required. Obtain forms from:** http://web.jhu.edu/animalcare/Instructions%20for%20ordering%20sustained%20release%20buprenorphine
NSAID		
Carprofen (Rimadyl ®)	5-10 mg/kg SC, PO q 24 hr	For optimal analgesia, give NSAID and buprenorphine.
Meloxicam (Meloxicam ®)	1-2 mg/kg SC, PO q 24 hr	

NEONATAL MOUSE & RAT

Inhalant Anesthesia

Agent(s)	Dose	Comments/Reference(s)
Isoflurane	3-5% Induction 1-3% Maintenance	Administer via precision vaporizer and compressed oxygen or drop method. Good first choice.

Hypothermia Anesthesia:

Comments: When inhalant anesthesia is not available or cannot be used safety, hypothermia is a relatively safe and effective alternative to injectable anesthetics in altricial rodents up to 7 days old.
Induction: Place the pup in a latex/nitrile glove finger and immerse the glove finger in crushed ice and water (2-3°C or 35-37°F) up to the level of the head so that the head of the pup is visible. Anesthesia induction takes 5-8 minutes.
Procedure: Remove the pup from the ice bath and place on a re-freezable ice pack. A piece of gauze or cloth should prevent direct contact of the pup's skin with the freezable ice pack. Duration of anesthesia on an ice pack is 15 minutes maximum.

Hypothermia Recovery: Rapid warming should be avoided. Pups can be placed in a small incubator (32-35 °C or 90-95°F) for gradual warming over 20-30 minutes. Once warmed for this time, circulating warm water blankets can be used until mobile where complete recovery takes 30-60 minutes. Once mobile, pups may be mingled with the litter to aid in covering the procedure odors on the pup then the litter returned to the dam.

Injectable Anesthesia: In general, injectable anesthetics are not as safe as hypothermia or isoflurane in neonatal rodents <6-7 days old. Several of the injectable combinations used in adult rodents have been found to be unpredictable and associated with >50% mortality rate. If injectable combinations are used, it is important to begin at the low end of the recommended dose range based on weight and only use the IP route. Also, the recovery period may be prolonged and hypothermia must be avoided by keeping the neonate warm as noted above.

Johns Hopkins University
Animal Care and Use Committee

Use of Neuromuscular Blocking Agents[1]

BACKGROUND: Federal regulations and policies state that: a) "Procedures that may cause momentary or slight pain or distress to the animal will not include the use of paralytics without anesthesia"[2]. b) "Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents." [3].

POLICY: Neuromuscular blocking agents such as succinyl choline, gallamine, and pancuronium are not to be used alone for surgical restraint or in painful procedures. They can be used for surgery or painful procedures only in conjunction with drugs known to produce adequate anesthesia/analgesia.

The use of a neuromuscular blocking agent in an animal manipulation will require a justification with extensive detail on the instrumentation/procedures used for determining that adequate analgesia is maintained. Some ways to determine that animals are adequately anesthetized include performing the procedure in absence of the neuromuscular blocker to determine appropriate anesthetic dosages and monitoring blood pressure and heart rate. Both the upper and lower range of acceptable blood pressure and heart rate values should be specified in the proposal. The parameters of any other signs (e.g. pupil size) that might be used for anesthetic depth assessment also should be specified. These values should be determined before the neuromuscular blocking agent is administered[4] (e.g. during pre-surgical period, in pilot studies, from previous experimental procedures not involving neuromuscular blocking agents or from the literature). Study records should document maintenance of adequate levels of anesthesia and analgesia during the use of neuromuscular blocking agents.

When neuromuscular blocking agents are used for experimental procedures involving more than a single short event, peripheral nerve stimulation tests are recommended to document recovery of neuromuscular function during the non-paralyzed periods of the anesthesia depth assessment.

---

[1] Approved by the Animal Care and Use Committee: September 19, 2002, reviewed and refs. updated September 25, 2012; reviewed 1/31/18.
[2] 9 CFR Chapter 1 Subchapter A Part 2 § 2.31 (d)(iv)(C)
[3] U.S. Government Principles for the Utilization and Care of Vertebrate Animal Used in Testing, Research and Training. Principle V. As cited in the Public Health Service Policy on Humane Care and Use of Laboratory Animals.
[4] Guide for the Care and Use of Laboratory Animals. NRC. National Academy Press, 8th ed., 2011. p123.

If animal subjects are to recover from the experimental procedures clinical signs of neuromuscular recovery should complement the nerve stimulation tests (e.g. normal breathing pattern, sustained head lift, or ability to stand).

Veterinary consultation is required in preparation of protocols requiring use of neuromuscular blocking agents[5].

A useful editorial article was published in *Anesthesiology* 85: 697-699; 1996.

[5] The use of neurotoxins may be subject to additional regulations by Departments of Health and Human Services (HHS) and Agriculture (USDA) and will require approval by the Biosafety Officer.

APPROVAL OF USE OF SUSTAINED RELEASE FORMULATIONS OF BUPRENORPHINE HCL IN CURRENTLY APPROVED PROTOCOLS[1]

PURPOSE:
This document provides recommendations on the replacement of buprenorphine HCl with sustained release formulations of buprenorphine. This guidance has been prepared under the direction of the JHU Attending Veterinarian, Dr. R. J. Adams, and approved by the JHU Animal Care and Use Committee. Any currently approved protocol that includes the provision of buprenorphine HCL for analgesia may substitute extended-release buprenorphine according to the guidelines below without first submitting an amendment for approval by the ACUC. An email must be sent to ACUC@jhmi.edu giving the protocol number and stating that use of extended release buprenorphine is being added to the protocol. The Principal Investigator must either send the email or be cc'd on it. New or 3rd-year renewal protocols should include use of the extended release buprenorphine as an option if buprenorphine is to be included as an analgesic.

BACKGROUND:
Buprenorphine SR and SR Lab (ZooPharm, Fort Collins, CO) are formulations that provide a longer duration of analgesia (potentially up to 72 hours) following administration of a single dose. The availability of these products creates the opportunity for refinement of current analgesia protocols

RECOMMENDATIONS:
Each dose of extended-release buprenorphine may be substitute for 48 hours of previously approved use of buprenorphine HCl. For example, in a protocol previously approved for 5 days of post-operative buprenorphine given every 8-12 hours, 3 doses of extended release buprenorphine would be required at 0, 48, and 96 hours.

Buprenorphine SR and SR Lab (Zoopharm; http://wildpharm.com/zoopharm-home.html)
Two concentrations are available: 1 mg/ml for small animals such as rodents (SR Lab) and 3 mg/ml for larger animals (SR).

Species	Recommended Dose	References
Mouse	0.5-1.0 mg/kg SC, q48 h	Carbone *et al.* 2012, Clark *et al.*, 2014
Rat	1.0-1.2 mg/kg SC, q72 h	Foley *et al.* 2011, Chum *et al.* 2014
Macaque	0.2 mg/kg SC, q48 h	Nunamaker *et al.* 2013
Pig	0.12 mg/kg SC, q48 h	Hanks *et al.* 2014
Cat	0.12 mg/kg SC, q48 h	Catbagen *et al.* 2011
Dog	0.06 mg/kg SC, q48h	Zoopharm, http://www.srvet.net/index.php/other/buprenorphine-sr/35-main/main/95-buprenorphinesrdosage

REFERENCES (additional information is available on each company's website provided above):

Carbone ET, Lindstrom KE, Diep S, and Carbone L. 2012. Duration of action of sustained-release buprenorphine in 2 strains of mice. Journal of the American Association for Laboratory Animal Science 51 (6): 815-819.

Catbagan DL, Quimby JM, Mama KR, Rychel JK, Mich PM. 2011. Comparison of the efficacy and adverse effects of sustained-release buprenorphine hydrochloride following subcutaneous administration and buprenorphine hydrochloride following oral transmucosal administration in cats undergoing ovariohysterectomy. Journal of the American Veterinary Medical Association 72 (4): 461-466.

Chum HH, Jampachairsri K, McKeon GP, Yeomans DC, Pacharinsak C, Felt SA. 2014. Antinociceptive effects of sustained-release buprenorphine in a model of incisional pain in rats (*Rattus norvegicus).* Journal of the American Association for Laboratory Animal Science 53 (2): 193-197.

Clark TS, Clark DD, Hoyt Jr. RF. 2014. Pharmacokinetic comparison of sustained-released and standard buprenorphine in mice. Journal of the American Association for Laboratory Animal Science 53 (4): 387-391.

Foley PL, Liang H, Crichlow AR. 2011. Evaluation of a sustained-release formulation of buprenorphine for analgesia in rats. Journal of the American Association for Laboratory Animal Science 50 (2): 198-204.

Hanks BC, Schlink S, Brown LD, Luna M, Liu J, Stricker-Krongrad A, Bouchard GF. 2014. Short-acting and long-acting buprenorphine therapeutic drug levels following single subcutaneous administration in diabetic Yucatan miniswine. Abstract in: International Journal of Toxicology. 33 (1): 65

Nunamaker EA, Halliday LC, Moody DE, Fang WB, Lindebald M, Fortman JD. 2013. Pharmacokinetics of 2 formulations of buprenorphine in macaques (*Macaca mulatta* and *Macaca fascicularis*). Journal of the American Association for Laboratory Animal Science 52 (1): 48-56.

[1] Approved by the Animal Care and Use Committee 9/14/14, revised 5/20/22

Johns Hopkins University
Animal Care and Use Committee

GUIDELINES ON ANALGESIA FOR RODENT SURGERIES

OBJECTIVE: To establish the minimum required analgesic regimens for rodent survival surgeries.

SCOPE: This applies to all rodents under the Johns Hopkins University animal care and use program. Exemptions to the minimum requirements may be approved with appropriate scientific justification and must be described in the ACUC-approved protocol.

PAIN CATEGORIZATION: The following provides general guidelines in the determination of the severity of pain associated with surgical procedures in rodents.

· Minimal to mild pain: Includes procedures that cause momentary pain or pain of low intensity that does not have long-lasting consequences.

· Mild to moderate pain: Procedure that cause more than momentary pain, and are known to be painful in humans hours to days after the procedure/surgery is performed. Would cause rodents to be visibly painful by displaying any one of the following behaviors if no analgesics were given (weight loss, decreased grooming, decreased activity, dark red material around the eyes of rats, hunched posture).

· Moderate to severe pain: Any procedure that causes intense pain, or a moderate pain that last days to weeks after the procedure is completed. This may include any surgery that induces a chronic pain typically associated with degenerative diseases (e.g., osteoarthritis).

GUIDELINES:

1. Determine pain categorization and appropriate analgesic regimen of the surgical model using the table below, which is not intended as a comprehensive list of procedures that fall into these categories.
2. Upon conducting animal experiments, provide analgesics in addition to the minimum described in the table below considering factors such as a) personnel's surgical experience and technique, and b) intra-and post-operative complications. For example, aggressive tissue handling and complications like accidental organ perforation may elevate the pain level each animal experience.
3. Assess the animal at least daily for 7-10 days post-operatively, or at least until the sutures, wound clips, or staples are removed.
4. Manage post-operative complications (e.g., suture dehiscence and wound infection) as described in the ACUC-approved protocol, or upon veterinary consultation. Administer analgesics as appropriate.
5. Consider analgesic adjuvants such as sedatives and adjunct pain management approaches like using soft bedding material (e.g., paper vs. corncob) to further alleviate pain and distress.

MINIMUM ANALGESIA REQUIREMENTS[1]			
	Minimal to mild pain	**Mild to moderate pain**	**Moderate to severe pain**
Pre-emptive analgesia[2]	Single dose of systemic NSAID (e.g. meloxicam or carprofen) **OR** Opioid (e.g. buprenorphine) prior to surgery	Systemic NSAID (e.g. meloxicam or carprofen) **OR** Single dose of buprenorphine SR[3] prior to surgery	Systemic NSAID (e.g. meloxicam or carprofen) **AND** Single dose of buprenorphine SR[3]
Intra-operative analgesia			Lidocaine and bupivacaine
Post-operative analgesia[4]	PRN	NSAID q 24h for 1 additional day (not necessary if buprenorphine SR[3] was administered pre-emptively) PRN after 1 day post-op	NSAID q 24h for 2 additional days PRN after 2 days post-op
EXAMPLES OF RODENT PROCEDURES			
	Subcutaneous osmotic pumps	Minor laparotomy (skin and muscle incision only - e.g., intra-peritoneal osmotic pump)	Major laparotomy (e.g., includes incising of viscera)
	Simple skin incision/biopsy	Craniotomy with significant tissue manipulation[5]	Middle cerebral artery occlusion
	Vascular cut-down	Ovariectomy	Meniscectomy
	Vasectomy	Orchidectomy	Carotid ligation
	Intracranial injection	Neural electrode implantation	Orthopedic procedures
			Hind limb transplant
			Thoracotomy

[1]Additional analgesics and other pain-relieving methods (e.g. local anesthetics) should be considered dependent on the expected outcome of the surgical procedure and/or in consultation with veterinary staff.
[2]Consider the surgery start time so as to reach therapeutic levels when animal recovers from anesthesia (e.g., 1h with injectable meloxicam [Chen et al., 2016], 2h with oral meloxicam or carprofen, and 12h with carprofen in drinking water [Ingrao et al., 2013]).
[3]The therapeutic duration of buprenorphine SR is 48h in mice and 72h in rats [Kendall et al., 2014; Foley et al., 2011]. Please note that meloxicam SR, carprofen-SR, fentanyl-SR, and butorphanol-SR do not provide analgesia for more than 24 hours [Kendall et al., 2014].
[4]Animals must be assessed daily (see item Procedures 3 above) and analgesics given PRN (*pro re nata*; "as needed").
[5]Craniotomy is defined as a surgical procedure used to temporarily open part of the skull to expose the brain. Examples of procedures that cause mild to moderate pain include cranial window and head cap placement.

SELECT REFERENCES:

1. **Chen, P.H., Boyd, K.L., Fickle, E.K., Locuson, C.W. 2016. Subcutaneous meloxicam suspension pharmacokinetics in mice and dose considerations for postoperative analgesia. *J Vet Pharmaco Ther*. 39: 356-362.**
2. **Foley, P.L., Liang, H., Chrichlow, A.R. 2011. Evaluation of a sustained-release formulation of buprenorphine for analgesia in rats. *JAALAS*. 50: 198-204.**
3. **Ingrao, J.C., Johnson, R., Tor, E., Gu, Y., Litman, M., Turner, P.V. 2013. Aqueous stability and oral pharmacokinetics of meloxicam and carprofen in male C57BL/6 mice. *JAALAS*. 52: 553-559.**
4. **Kendall, L.V., Hansen, R.J., Dorsey, K., Kang, S., Lunghofer, P.J., Gustafson, D.L. 2014. Pharmacokinetics of sustained-release analgesics in mice. *JAALAS*. 53: 478-484.**

Johns Hopkins University
Animal Care and Use Committee

Euthanasia of Mice and Rats Using Carbon Dioxide[1]

PURPOSE: This document provides guidance on the correct procedures to follow when euthanizing mice and rats using carbon dioxide.

BACKGROUND: Euthanasia of animals at Johns Hopkins University must be carried out according to the most recent guidance of the American Veterinary Medical Association (AVMA).[2] Carbon dioxide (CO_2) inhalation is a common method of euthanasia used for rats and mice. It is the method that will be used by central facilities staff with mice and rats identified for euthanasia by researchers. Use of CO_2 euthanasia by researchers must be included in an ACUC-approved protocol. Appropriate technique, equipment, and source of CO_2 must be used. Compressed CO_2 gas in cylinders is the only approved source because the flow of gas to the euthanasia chamber can be regulated precisely**.** The practice of immersion, where conscious rodents are placed directly into a container prefilled with 100% **CO_2**, is unacceptable. **CO_2 generated by other means such as dry ice, fire extinguishers, or chemical means (e.g., antacids) is also unacceptable.**

Upon completion of the procedure, death must be confirmed for each animal by one or more of the methods listed below, as approved in the ACUC protocol. It is important to understand that short-term CO_2 exposure produces anesthesia. So failure of the animal to move or show a reflex response is not sufficient to confirm death. **Disposal of an anesthetized, rather than a euthanized, animal is a serious animal welfare concern.** Understanding how to avoid this is the responsibility of anyone carrying out euthanasia with CO_2.

PROCEDURES: For euthanasia requests to animal care staff: Do not combine animals from different cages and do not leave pups under 21 days of age in the cage without the dam.

Follow the guidance below when euthanizing animals yourself.

1. Preferably, euthanize animals in the home cage to minimize the stress of being placed into an unfamiliar enclosure and to prevent social aggression. Less ideally, a single adult mouse, 1 litter, or up to 3 weanlings can be euthanized in a critter carrier (small cardboard container). Animals in the euthanasia chamber should be readily visible. All animals in the chamber must be able to make normal postural adjustments.

---

[1] Approved by the JHU Animal Care and Use Committee on: April 17, 2003---revised 9/28/2006, 7/17/08, 10/15/18, 8/20/2020, 9/17/2020.

[2] AVMA Guidelines on Euthanasia of Animals: 2020 Edition. Available at www.jhu.edu/animalcare/

2. If euthanizing selected animals in a social group, transfer the animals to be retained to a new cage; and euthanize the intended animals in the home cage.

3. On occasion, it may be useful to hold more than 5 mice per cage (e.g., mice being collected for immediate euthanasia). This is acceptable but only as long as the following conditions are met:
    a. Up to 10 compatible mice may be placed in a temporary holding cage for up to 30 minutes, but only if the holding cage is not left unattended.
    b. If fighting is observed, immediately separate the animals.
    c. Adult males ≥ 6 weeks old from different cages should not be combined.
    d. If **only** euthanizing pups less than 10 days of age, up to 2 litters may be combined in a single cage.

4. Without prefilling the chamber, place the animals in the chamber and introduce 100% CO_2 at a displacement rate of 30-70%. Humane application of CO_2 requires the use of a pressure-reducing regulator and flow meter that will generate the recommended displacement rates. To ensure the chamber flow rate displaces at least 30% - 70% of the chamber volume per minute, follow "Operation of **CO_2** Station" instructions posted near the chamber. Animals should be exposed to the CO_2 for the applicable duration listed in the table below.

Exposure Time Chart

Age of Animal	Time of gas flow	Time of continued exposure	Total of time exposure
10 day to adult mice	2-4 minutes	3-6 minutes	5-10 minutes
10 day to adult rats	2-4 minutes	5-10 minutes	7-14 minutes
Newborn to 10 days old pups: CO_2 exposure only*	**2-4 minutes**	**48-50 minutes**	**50-54 minutes**
Newborn to 10 days old pups: CO_2 exposure immediately followed by secondary method.**	**2-4 minutes**	**5-10 minutes**	**7-14 minutes**

***Neonates are resistant to the hypoxia-induced effects of CO_2. Thus, CO_2 exposure time must be considerably longer if a secondary method, such as decapitation, is not performed.**
****Under the AVMA 2020 guidance, secondary methods, such as decapitation, may only be performed after the neonate is nonresponsive to painful stimuli (e.g., a toe pinch).**

5. After exposure for the appropriate period of time, verify that each animal is dead. Use the method stated in question 16d of your ACUC protocol to confirm death (see below for examples). Any animals with signs of life (e.g., gasping) must be returned to the CO_2 chamber; or, if approved in your protocol, a secondary method of euthanasia (see list below) may be quickly performed.

6. When disposing of the carcasses, place them in a red biohazard bag. Put the bag(s) in a designated carcass refrigerator or freezer.

Methods of confirming death that may be included in the ACUC-approved protocol

1. Use visual and physical examination to verify that the heart has stopped beating and the animal is not breathing; mucous membranes should be pale or white.
2. Observation that the animal fails to recover within 10 minutes after CO_2 exposure ends.

Secondary methods for completing euthanasia that may be included in the ACUC-approved protocol

3. Cervical dislocation
4. Decapitation
5. Immediate harvest of vital organs (i.e., heart, lungs, or brain)
6. Bilateral thoracotomy (pneumothorax)
7. Exsanguination