GUIDELINES FOR THE UTILIZATION OF ANESTHETICS AND ANALGESICS IN SMALL LABORATORY ANIMALS

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Physiologically, pain is a stressor, and if not alleviated, can potentially lead to unacceptable levels of distress in animals. Federal laws and regulations require that pain and discomfort be avoided in experimental animals by utilizing appropriate anesthetic and analgesic agents during and after surgery or painful experimental manipulations. The *Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals* specifically states that, "procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator."[45] In addition, the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* states, "proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative."

The appropriate use of anesthetic and analgesic agents, as well as investigator experience with their administration, is carefully examined as part of the protocol review process conducted by the Institutional Animal Care and Use Committees (IACUCs) at the Memorial Sloan Kettering Cancer Center, Weill Cornell Medicine, and the Hospital for Special Surgery. The Research Animal Resource Center's (RARC) Veterinary Services staff should be consulted for assistance in designing appropriate anesthetic and analgesic regimens for laboratory animals, and they are available for assistance with training and/or the administration of anesthetic agents. The use of any anesthetic or analgesic agent must be approved by the IACUC prior to administration, unless administered under the direction of a RARC veterinarian.

The Office of Laboratory Animal Welfare (NIH) states that "pharmaceutical-grade substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and/or interfere with the interpretation of research results." A pharmaceutical-grade chemical or compound meets the standards of purity and composition established by the United States Pharmacopeia National Formulary (USP/NF) or the British Pharmacopoeia (BP). Non-pharmaceutical-grade chemical compounds can only be used in animals after specific review and approval by the IACUC. For further details, please consult the *IACUC Policy on the Use of Non-Pharmaceutical-Grade Compounds*.

General Considerations

The ability to recognize clinical signs of pain is essential in order to provide appropriate pain relief. However, significant variability in responses to painful stimuli is observed between species, and even among individuals within a given species. Despite these differences, unless the contrary is known or established, it should be assumed that procedures that cause pain in humans might also cause pain or distress in other animals.[26]

In some instances, animals may not respond to conditions and procedures that cause pain in humans in a manner that is immediately apparent as a pain-related behavior. For example, among prey species (mice, gerbils, rats, guinea pigs, hamsters, etc.) suppression of overt pain behaviors may have important survival implications in their natural habitat. Expression of evolutionary

behavior is also common in laboratory animal species, and the investigative staff member who is unfamiliar with a given species may not be able to distinguish what is, or is not, normal behavior and thus pain may go unrecognized.[1]

Distinctions between observed behaviors of animals, and that which would be predicted from human patients, often lead to the assumption that although pain may occur in animals, they do not require analgesics as frequently as human patients and perhaps may not require them at all. Based on extensive physiologic and behavioral analyses, these assumptions have been proven false. Rather, it has been shown that efficacious postoperative analgesia may decrease morbidity and reduce mortality following surgical manipulations.[1] Moreover, poor pain management can potentially invalidate research results, as uncontrolled pain may initiate a stress response, resulting in hormonal, metabolic and physiologic imbalances.[1]

While it is unrealistic to propose that analgesic compounds be administered to experimental animals in all circumstances, it is important that a *realistic and reasoned* assessment be made for the need to control pain, distress, inflammation, or febrile responses, and the possible contraindications of such treatment.

General Principles

When initiating an experiment involving anesthetics, the following considerations and practices should be carefully reviewed and implemented:

a. Animals should not express signs of illness prior to undergoing a procedure requiring anesthesia, as induction and recovery may be compromised. Consider age, health, nutritional, reproductive, and experimental status prior to initiating anesthesia. Animals should be acclimated to the facility for at least 72 hours prior to undergoing surgical procedures.
b. It is generally unnecessary, nor advisable, to withhold food and water from most rodents prior to anesthesia. If fasting is warranted for a specific procedure (i.e., colonoscopy), it must be described in the IACUC protocol.
c. Consider administering a parasympatholytic agent (atropine or glycopyrrolate) 15-30 minutes prior to anesthesia to reduce respiratory secretions and prevent bradycardia.
d. Monitor the animal's vital signs at least every 15 minutes during anesthesia.
e. If an animal is responsive to a noxious stimulus following the initial dose(s) of anesthetic/sedative agent(s), additional anesthetics should be given as described in the IACUC protocol.
f. Anesthetized animals may not breathe effectively. Intubate anesthetized animals whenever possible or consider providing supplemental oxygen via a nose cone. Intubation is generally required for procedures involving the thoracic cavity.
g. Animals should be kept warm while anesthetized as hypothermia will slow clearance of the anesthetic and may result in increased recovery times or mortality. To prevent burns, animals must not be placed directly on a heat source.
h. Apply sterile eye lubricant to both eyes to prevent corneal drying. Not required if the duration of anesthesia will not exceed 5 minutes.
i. Ensure that you have sufficient anesthetic and analgesic drugs at your disposal, especially if you plan to perform multiple procedures. Emergency drugs and equipment as well as

reversal agents, if necessary, should be readily available and personnel should be knowledgeable in their use.

j. Pre-emptive analgesia may be required prior to starting a procedure. Consult your IACUC protocol.
k. RARC's veterinary and technical staff is available to assist with planning and in an emergency. This may be arranged by sending an email to rarc_vs@med.cornell.edu (WCM/HSS) or rarc_vs@mskcc.org (MSK). It is the responsibility of the research staff to immediately contact RARC personnel if complications arise. Please call Veterinary Services at 212-746-1079 (WCM) or 646-888-2430 (MSK) if the issue arises during normal business hours. Outside of normal business hours, please call 212-746-1022 and request to have the Veterinarian-On-Call paged.
l. There may be considerable individual, sex, and/or strain variations in response to the administration of anesthetics or analgesic agents. It is therefore essential to assess anesthetic doses and analgesic effects in each set of animals or conduct pilot studies for inbred or mutant strains whenever possible.

Anesthesia

Anesthesia is a reversible process that produces central nervous system depression to prevent spontaneous and conscious movement. It is used to produce a convenient, safe, and effective means of inducing a physiological state that allows clinical and/or surgical procedures to be conducted with a minimum of stress, pain, discomfort, or toxic side effects to the animal or to the anesthetist.

Properties of an ideal anesthetic agent:

Provides anesthesia (loss of sensation in a body part, or the whole body) and analgesia (loss of sensitivity to pain) and:

a. Is not toxic to the animal and surgical personnel;
b. Produces smooth induction and recovery;
c. Has predictable physiologic effects; and,
d. Is readily available.

The following criteria should be critically evaluated when selecting an anesthetic agent for research use:

a. Species utilized;
b. Nature of the procedure (acute/terminal versus chronic/survival; minor versus major);
c. Duration of anesthesia required;
d. Ease of administration;
e. Physiologic effects;
f. Safety concerns;
g. Reversibility;
h. Degree and duration of analgesia required;
i. Recovery characteristics of the agent(s); and,
j. Potential effects on research aims.

Note: Many anesthetic agents have been shown to produce undesirable physiologic effects that may preclude their use in specific research applications. Thus, investigators are encouraged to familiarize themselves with the pharmacology of the various agents proposed in their research studies prior to implementation. The *"Guidelines for the Assessment and Management of Pain in Rodents and Rabbits*," developed by The American College of Laboratory Animal Medicine includes a summary of physiologic effects associated with the use of opioids and non-steroidal anti-inflammatory agents.[1]

Anesthetic Monitoring

Anesthetic depth: Anesthetized animals must be monitored throughout the procedure to assure that they remain at the appropriate anesthetic "plane" (see below) and are not too light or deep. Animals should remain in stage 3 of anesthesia for the duration of the procedure. It is important to visually evaluate the animal and not rely solely on monitoring instruments as instruments can malfunction. Respiratory pattern and frequency, as well as mucous membrane color and capillary refill time, are easily monitored in rodents and are good indicators of anesthetic depth. Given the small size of these patients, attention should be paid to both thoracic and abdominal excursion/movement for assessment of respiratory rate. The color of the mucous membranes (lips, gums, tongue, vulva, prepuce, etc.) and extremities (ears, digits, tail) should be pink, and not dusky grayor blue as this reflects cardiopulmonary compromise. Capillary refill time is a good indicator of cardiovascular function and can be assessed by briefly applying gentle pressure to an area of accessible mucous membrane (gums & conjunctiva) with a finger, relieving pressure, and determining the time until the blanched tissue returns to normal color. Typically, capillary refill time in a healthy anesthetized animal is between 1-3 seconds. Prolonged refill time (>3 sec.) is suggestive of cardiovascular compromise, including excess fluid loss, anesthetic narcosis, and/or hypotension.

Assessment of Anesthetic Depth in Rodents

	Respiratory rate and effort	Pulse Quality	Pedal or Tail reflex	Palpebral Reflex
Stage 1 - Induction	Smooth	Strong	Present	Present
Stage 2 – Excitement	Increased	Strong tothready	Present	Present
Stage 3 – Surgical	**Regular, shallow**	**Decreased,but regular**	**Absent**	**Absent**
Stage 4 – Overdose	Absent	Absent	Absent	Absent

Reflexes: One or more of the following reflexes should be evaluated prior to initiation of surgery, and every 5-10 minutes thereafter during the surgical procedure. There may be significant differences in the time of loss of specific reflexes,and thus it is recommended to test all the reflexes listed below before beginning a potentially painful procedure or exposure to other noxious stimuli (e.g., placement in a stereotactic unit with ear bars).

- Righting reflex – place the animal on its back and confirm that no spontaneous postural correction occurs.
- Pedal reflex (toe pinch) or tail pinch reflex – firmly pinch a single digit of the hindlimb or the tip of the tail and confirm the absence of spontaneous movement or reaction/withdrawal.

- Palpebral reflex – gently apply a cotton tip applicator to the eyelids at the medial or lateral canthus and confirm that no blinking occurs.

Note: Ear twitch or paddling of the legs may occur spontaneously, and thus may not be a reliable indicator of anesthetic depth in certain species. For example, a paddling reflex is often present in hamsters, and the pedal reflex may be present in guinea pigs despite adequate anesthesia. Vocalization is a sign of improper anesthetic depth.

Anesthesia Reversal and Recovery

In order to avoid complications associated with prolonged anesthesia, such as hypothermia, dehydration, and hypoglycemia, reversal agents may be administered to hasten the time it takes for animals to recover from anesthesia and resume normal activity. These agents can also be used to reverse the detrimental effects of anesthetic agents in the event of an overdose. Reversal agents are commonly available, particularly for alpha-2 adrenoreceptor agonists such as xylazine and dexmedetomidine. Atipamezole (Antisedan®) is a synthetic alpha-2 adrenergic agonist that competes for binding sites on both central and peripheral receptors in order to reverse neurological, cardiovascular, and respiratory depression. *Janssen et al.* report that the time to return to righting reflex when using ketamine-xylazine anesthesia is significantly lower following the administration of atipamezole (approximately 10 minutes) than without the administration of a reversal (approximately 40 minutes). It is important to note that reversal agents should not be used alone, and the effects of other anesthetic agents administered will not change. For example, if using ketamine-xylazine anesthesia with opioid analgesia, the anesthetic effects of ketamine and analgesic effects of the opioid will remain, while the effects of xylazine will be reversed if the appropriate dose of atipamezole is given.

The timing of atipamezole administration is important. Administering atipamezole too early, i.e., 10 minutes after induction of anesthesia with ketamine-xylazine has been shown to increase time to resuming normal walking activity in mice. If you choose to use a reversal agent, only administer the drug once anesthesia is no longer needed. Make sure you have completed the procedure, provided analgesia, and are ready for the animal to recover.

Whether or not you elect to use a reversal agent, it is imperative that you monitor the animal's recovery from anesthesia until they are fully awake and able to maintain sternal recumbency. Animals may be recovered on a paper towel placed within a cage or a cage without bedding to prevent aspiration of bedding material. A RARC approved heating pad may be placed underneath 50% of the cage for added temperature support.

Animals should not be returned to the animal holding room until they are fully recovered from anesthesia and can ambulate normally. If a paper towel is used, it must be removed from the cage prior to returning the animal to the holding room.

If you have any concerns regarding an animal's recovery from anesthesia, please call Veterinary Services at 646-888-2430 (MSK) or 212-746-1079 (WCM) if the issue arises during normal business hours. Outside of normal business hours, please call 212-746-1022 and request to have the Veterinarian-On-Call paged.

Analgesia

Pain is an anticipated outcome of surgical manipulation and the appropriate use of analgesic agents in rodents and small laboratory animals is an important component of the humane use of these species. The use of analgesics should be considered in all animal studies where experimental manipulations may result in more than transitory pain. Preemptive analgesia is the preferred method of pain control.

Preemptive analgesia is the provision of pain-relieving agents prior to initiation of a painful procedure to prevent "wind-up" (persistent changes in the excitability and sensitization of neurons in the pain perception pathway) leading to a painful response to a normally innocuous stimulus, referred to as allodynia, and also hyperalgesia, an increased response to a painful stimulus. To prevent these changes, analgesic agents are administered before the surgical incision is made and tissues are manipulated as a way to reduce immediate post-operative pain and avoid future chronic pain. This practice does not negate the need for post-operative monitoring and pain management. Additionally, preemptive analgesia may decrease anesthetic needs peri-operatively thereby decreasing side effects and required dose of these agents. Preemptive analgesia is utilized widely in human and veterinary medicine and is recommended by RARC as best common practices to improve animal welfare.

General considerations

a. Analgesics should be administered when painful procedures are performed, and whenever signs of pain and/or distress are detected. See common behavioral signs of pain in rodents below.
b. Analgesics are most beneficial when given prior (pre-emptively) to the onset of physiologic pain.
c. Analgesics given at the time of anesthetic induction typically result in the use of less analgesic, and for a shorter duration, postoperatively.
d. Some anesthetic combinations, such as ketamine/xylazine, will provide several hours (3-5 hours) of post-operative analgesia. As such, animals may not require additional analgesics for several hours post-surgery. If supplemental analgesics are administered in the immediate post-operative period, the dose should be adjusted accordingly.
e. Isoflurane is a good inhalant anesthetic agent. However, because it is minimally metabolized and rapidly expelled from the airway, there is no residual analgesic effect once the animal awakens. Therefore, analgesics should be administered before recovery when painful procedures are performed when isoflurane is used as the primary anesthetic.

Behavioral Signs of Pain in Rodents[1]

Mild signs	Lack of grooming Weight loss Mentation, immobile but alert
Moderate signs	Piloerection Hunched posture Squinted eyesLameness
Severe signs	Inactive Eyes closed Weight loss Tachypnea
General signs	Decreased/absent nest buildingAltered group behavior Cannibalism of offspring

A more objective and accurate method of determining if a mouse or rat is exhibiting pain is to calculate a pain score. The mouse[34] and rat[54] Grimace Scoring System is recommended. This method evaluates facial cues including squinted eyes, cheek/nose bulge, and whisker/ear position to provide a score, which may indicate pain.

The following appendices provide supplemental information:

Appendix I contains a brief discussion of the pharmacology of select anesthetic and analgesic agents used in the research setting.

Appendix II describes anesthetic techniques commonly used in small mammalian species in the research setting.

Appendix III describes analgesic techniques and provides dosing regimens for small mammalian species in the research setting.

Appendix IV describes dilution instructions for common rodent anesthetics and analgesics.

[1] Adapted from Flecknell *et al*., 1996

APPENDIX I

Pharmacology of Select Anesthetic and Analgesic Agents

Acepromazine (AceproJect®, PromAce®, Generic):
A phenothiazine tranquilizer that produces CNS depression with sedation, muscle relaxation, and hypotension. It is frequently used as a preanesthetic in general anesthetic regimens to provide smoother induction of general anesthesia, thereby reducing the amount of general anesthetic required. Acepromazine provides synergistic sedative and analgesic effects when combined with opioids.

Alpha-2 Adrenoceptor Agonists: Xylazine (Anased®, Xylamed®, Generic) and Dexmedetomidine (Dexdomitor®, Generic):
Drugs within this class are non-narcotic and result in sedation and antinociception with muscle relaxant and analgesic properties. These agents are occasionally used alone as a tranquilizer, but are most often used in combination with ketamine to provide general anesthesia in a number of small animal species. These agents bind to alpha-2 adrenergic receptors making them unresponsive to excitatory stimuli via activation of G-protein coupled receptors, and open potassium channels in neuronal cell membranes. Examples of alpha-2 adrenoceptor agonists include xylazine and dexmedetomidine. Dexmedetomidine is more selective for the alpha-2 adrenoreceptors and is characterized by faster elimination when compared to xylazine.

These agents cause a decrease in heart and respiratory rates and an increase in peripheralvasomotor tone, which causes mucous membranes to appear blue-gray after administration followed by hypotension via stimulation of central receptors and depression of vasomotor tone. Though they cause skeletal muscle relaxation, they have been shown to increase intrauterine contractions and pressure and should be used with caution in pregnant animals. The routine use ofanticholinergics simultaneously with or after dexmedetomidine administration is not recommended as it could lead to adverse cardiovascular effects.

In the event of an accidental overdose, cardiac arrhythmias, hypotension, and profound CNS and respiratory depression can occur. In such instances, a reversal agent must be administered to prevent death of the animal. Atipamezole can be used to reverse any alpha-2 adrenoceptor agonist, as it is an antagonist that competes for receptor binding sites (see below for additional information). Xylazine was previously available in 2 concentrations (20 and 100 mg/ml); but manufacturing of the lower 20 mg/ml formulation was recently discontinued. Therefore, it is important that you confirm the concentration of xylazine when preparing cocktails to ensure animals are dosed appropriately. Cocktail recipes for both restraint and surgical anesthesia are provided in Appendix IV. Xylazine, when given in combination with ketamine, may affect glucoregulatory hormone levels in rats depending on the animal's fasted or fed state. These interactions must be considered when selecting anesthetic agents in animals with diabetes or other models where glucose and/or glucoregulatory hormone levels may affect the outcome.[51]

Alpha chloralose:
A hypnotic that provides minimal analgesia which is used alone or in combination with urethane to produce anesthesia of long duration. This agent may only be used for specific applications and must be scientifically justified in an approved IACUC protocol. It is prepared by heating the powdered drug in water at 60°C to form a 1% solution. Ideally, the animal should be premedicated

with another agent because marked excitement can occur during induction and painful manipulations should be carried out under a more effective anesthetic prior to maintenance with alpha chloralose. It can only be used for **non-survival** procedures. Alpha chloralose is a hazardous chemical and the *IACUC Policy on the Use of Non-pharmaceutical Grade & Compounded Pharmaceutical Grade Substances* must be adhered to when using this agent. Typical doses for chemical restraint are 85 mg/kg IP in mice and 45-55 mg/kg IP in rats for providing an 8-10 hr duration.

Atipamezole (Antisedan®):

Atipamezole is a synthetic alpha-2 adrenergic antagonist and thus competes with agonists for binding sites on both central and peripheral receptors, reversing neurological, cardiovascular, and respiratory depression associated with agonist administration. It may cause muscle tremors if given alone. Its use is not required following the administration of alpha-2 adrenergic agonists, but it can be used to prevent prolonged recovery or in the event of an overdose or adverse reaction to anesthesia (e.g., respiratory arrest).

Atropine (Atroject®, Generic):

A parasympatholytic agent, which antagonizes acetylcholine at its receptors. Atropine is an integral part of a general anesthetic regime. It is given preoperatively to decrease respiratory secretions and prevent bradycardia and cardiac arrhythmias associated with manipulation of the eye.

Bupivacaine (Marcaine®, Generic):

A long-acting local anesthetic that prevents nerve impulses by inhibiting passage of sodium ions through ion-selective channels in nerve membranes. Decreased permeability to sodium slows the rate of depolarization such that a threshold potential is not achieved and therefore an action potential is not propagated. Local anesthetics can markedly reduce requirements for general anesthetics. Combining bupivacaine with opioids can result in an enhanced and prolonged duration of hypoalgesia (reduced pain). Bupivacaine can cause cardiovascular toxicity following IV administration so this route is contraindicated. The duration of local anesthesia variesby species from 1-2 hours in rodents to 4 hours in other species.

Buprenorphine (Buprenex®, Ethiqa XR®):

A potent, *mu*-agonist-antagonist narcotic, which has marked analgesic properties. Buprenorphine is the preferred analgesic for alleviation of moderate to severe post-operative pain in most laboratory species. Buprenorphine is a Schedule III controlled substance, therefore records of its purchase and use must be maintained as described in the EHS guidance document *Security of DEA Controlled Substances* (WCM), or the *Guidelines for Requesting, Using and Storing Controlled Substances* (Burke), or the *Policy on Controlled Substances in Laboratory and Animal Research* (MSK). Respiratory depression is a rare, but possible adverse effect. Sedation is also possible. Several pharmaceutical-grade preparations of buprenorphine are available requiring different dosages and which provide markedly different durations of action. Each dose of Buprenex® provides pain relief for 4-12 hours. Each dose of extended-release Ethiqa XR® provides pain relief for at least 48 hours in mice and 72 hours in rats. Ethiqa XR® is recommended because it provides consistent analgesic levels and requires less post-surgical animal handling. In general, extended-release medications require a longer duration of time until onset of action, therefore, Ethiqa XR® must be given before a painful procedure is conducted, i.e., at the time the mouse is anesthetized for surgery. Breakthrough pain is possible when using Ethiqa XR® and supplemental doses of

analgesics should be provided to address this. Adverse events, including death, have been reported when high doses of NSAIDS (e.g., meloxicam, carprofen) were administered concomitantly in mice. Therefore, only the doses recommended in this document should be used. Another dose of Ethiqa XR® should not be given until 48 hours (mice) or 72 hours (rats) after the first dose. If breakthrough pain occurs when using Ethiqa XR®, please contact a RARC veterinarian for guidance.

Carprofen (Rimadyl®):
A non-steroidal anti-inflammatory drug (NSAID) derived from propionic acid with potent anti-inflammatory and analgesic properties whose activity is likely mediated through its inhibition of cyclooxygenase. Use as an anti-inflammatory agent and/or when controlling mild to moderate pain. May be used in conjunction with an opiate to control severe pain.

Chloral hydrate:
A sedative/hypnotic that may be used only for terminal surgical procedures and must be scientifically justified in an IACUC approved protocol. It has a narrow margin of safety with severe respiratory, cardiovascular, and thermoregulatory depression. Chloral hydrate is a carcinogen and a hazardous chemical, and the IACUC Policy on the Use of Non-pharmaceutical Grade & Compounded Pharmaceutical Grade Substances must be adhered to when using this agent. Chloral hydrate is a Schedule IV controlled substance, therefore appropriate records must be kept as described in the EHS guidance document *Security of DEA Controlled Substances* (WCM), or the *Guidelines for Requesting, Using and Storing Controlled Substances* (Burke) or the *Policy on Controlled Substances in Laboratory and Animal Research* (MSK).Typical doses are 60-400 mg/kg IP in mice and 200-400 mg/kg IP in rats for 60-90 minute duration.

Diazepam (Valium®):
A benzodiazepine compound, which has anxiolytic, muscle relaxant, and anticonvulsant properties. It appears to act on parts of the limbic system, the thalamus and the hypothalamus to produce its calming effects. It can be used in laboratory animals as a tranquilizer, as a preanesthetic, or in combination with other drugs to produce general anesthesia. Diazepam is a Schedule IV controlled substance, therefore, appropriate records must be kept as described in the EHS guidance document *Security of DEA Controlled Substances* (WCM), or the *Guidelines for Requesting, Using and Storing Controlled Substances* (Burke) or the *Policy on Controlled Substances in Laboratory and Animal Research* (MSK).

Glycopyrrolate (Robinul-V®, Generic):
A synthetic quaternary ammonium compound that is used as an anticholinergic agent. As with atropine it is indicated as a preoperative agent to reduce salivary, tracheobronchial, and pharyngeal secretions. It also blocks the cardiac vagal inhibitory reflexes as described for atropine. In comparison to atropine, glycopyrrolate has a longer half-life, and has less of a cardioacceleratory effect.

Isoflurane (Forane®, Attane™, Generic):
A colorless, nonflammable, stable, liquid inhalant general anesthetic agent which has a characteristic mild pungent musty odor. Isoflurane is rapidly absorbed from the alveoli. It is rapidly distributed into the CNS and crosses the placenta. The vast majority of the drug is eliminated via the lungs; isoflurane is an inhalant anesthetic that has some distinct advantages over both halothane or methoxyflurane due to its lessened myocardial depressant and catecholamine sensitizing effects

and the ability to safely use it in patients with either hepatic or renal disease.

Ketamine (Ketaset®, Ketalar®, Generic):
A dissociative anesthetic with central nervous system (CNS) depressant activity without muscle relaxation. It has a wide margin of safety when administered IM. It is frequently usedin combination with other agents such as xylazine and diazepam during general anesthesia. Ketamine is occasionally used alone as a tranquilizer or for restraint, but it has limited analgesic effect and may produce cardiovascular stimulation. The eyes of animals receiving ketamine usually remain open and should be protected from injury and excessive drying of the cornea by use of a sterile ophthalmic lubricant. Ketamine is registered as a Schedule III controlled substance; therefore records of its purchase and use must be maintained as described in the EHS guidance document *Security of DEA Controlled Substances* (WCM), or the *Guidelines for Requesting, Using and Storing Controlled Substances* (Burke), or the *Policy on Controlled Substances in Laboratory and Animal Research* (MSK).

Ketoprofen (Ketofen®):
A non-steroidal anti-inflammatory drug (NSAID) derived from propionic acid with potent anti-inflammatory, antipyretic, and analgesic properties whose activity is mediated through its inhibition of lipoxygenase. Use as an anti-inflammatory/anti-pyretic agent and/or when controlling mild to moderate pain. May be used in conjunction with an opiate to control severe pain.

Lidocaine Jelly 2% (Xylocaine®, Generic):
A short-acting local anesthetic that prevents nerve impulses by inhibiting passage of sodium ions through ion-selective channels in nerve membranes. Decreased permeability to sodium slows the rate of depolarization so that threshold potential is not achieved and therefore an action potential is not propagated. Local anesthetics can markedly reduce requirements for general anesthetics. Lidocaine jelly (without prilocaine) has minimal anesthetic activity on intact skin. This is due to its low permeability through the skin and relatively high drug concentrations required for effective anesthesia. Therefore, this local anesthetic formulation is only effective on mucous membranes where the skin barrier does not exist.

Lidocaine/prilocaine cream (EMLA Cream®; lidocaine 2.5% and prilocaine 2.5%):
An emulsion indicated for use as a topical local anesthetic with each gram of EMLA cream containing 25 mg each of lidocaine and prilocaine. Application of EMLA cream should only be on normal intact skin as application to broken or inflamed skin may result in increased systemic absorption and toxicity. EMLA Cream should be applied 20-60 minutes before a procedure for maximal effect. The area should be covered with an occlusive dressing during this time to increase the absorption of the EMLA cream.

Meloxicam (Metacam®):
Meloxicam has anti-inflammatory, analgesic and antipyretic activity similar to other NSAIDs. Meloxicam exhibits its actions through inhibition of cyclooxygenase, phospholipase A2 and prostaglandin synthesis. It is considered COX-2 preferential (not COX-2 specific) as its COX-2 specificity is diminished at higher doses. This receptor preference decreases the risk of adverse gastrointestinal side effects. Meloxicam is available in honey-flavored syrup for oral (per os: PO) administration (1.5 mg/ml) and in an injectable form (5 mg/ml).

Meloxicam is not recommended for pregnant animals. Studies have shown that meloxicam may have adverse effects on the fetus via reduction of prostaglandin E synthesis resulting in a prolonged

gestation period and increased newborn mortality.

Sodium Pentobarbital (Nembutal®, Fatal Plus®):
An oxybarbiturate that is used to anesthetize a number of species. Pentobarbital tends to lower body temperature, heart rate, and respiratory rate and has a long duration of action and a narrow margin of safety in most species. Maintenance of body temperature is imperative with its use, as metabolism and the margin of safety decrease with lower body temperature. The duration of action of pentobarbital in rodents may depend on the type of bedding utilized and the time of day the animal is anesthetized. Soft wood bedding such as pine bedding induces hepatic microsomal enzyme systems that may affect pentobarbital pharmacokinetics (decreased duration of action due to increased metabolism). Pentobarbital is a Schedule II controlled substance and records of its purchase and use must be maintained as described in the EHS guidance document *Security of DEA Controlled Substances* (WCM), or the *Guidelines for Requesting, Using and Storing Controlled Substances* (Burke), or the *Policy on Controlled Substances in Laboratory and Animal Research* (MSK). If pentobarbital will be used for a survival procedure or a non- survival surgery, Nembutal® must be used. Nembutal® is currently the only commercially available product containing pentobarbital that is FDA approved and labeled for use as an anesthetic, and itis not always available. It is recommended that you discuss the use of this drug with a RARC veterinarian if you wish to use it for a survival or non-survival surgical procedure. If you wish touse pentobarbital for a procedure that results in the animal's irreversible death, such as perfusion,exsanguination, or tissue harvest, a dilution of a commercial product intended for animal euthanasia may be used for mice and rats (not for other rodents). Fatal Plus® is supplied as a 390mg/ml solution of sodium pentobarbital solution. Please note that other brands of euthanasia solution may contain other additives and should not be used.

2, 2, 2 Tribromoethanol (Previously known as Avertin):

Tribromoethanol (TBE) rapidly produces generalized central nervous system (CNS) depression, which extends to both respiratory and cardiovascular depression. TBE may only be utilized as a short-acting (approximately 20 minutes) general anesthetic for mice for specific applications, and must be scientifically justified in an IACUC-approved protocol. Use of this agent has been associated with intestinal ileus, peritonitis, hepatic and renal damage, and death as well as undesirable metabolic and physiologic derangements. Some of these adverse effects are dose and/or concentration-dependent and are more severe with the administration of additional doses. Therefore, the concentration of the injected solution cannot be greater than 1.5% and it can only be administered once to an animal for a survival procedure. If a mouse is not adequately anesthetized by the initial injection, the animal cannot be redosed and should either be euthanized or allowed to recover from anesthesia. TBE in the powdered form is a hazardous chemical and the *IACUC Policy for the Preparation and Use of Tribromoethanol in Mice* must be followed when using this agent.

Urethane:

Urethane, either alone or in combination with alpha chloralose (provides increased analgesia), may be used for procedures which require long-duration, cardiac-stable anesthesia. It may be used only for terminal surgical procedures and must be scientifically justified in an IACUC-approved protocol. It is purchased in the crystalline state and must be dissolved in water for IV or IP use. **Urethane is a mutagen and carcinogen**; therefore, nitrile gloves must be worn whenever handling any form of this hazardous compound. The *IACUC Policy on the Use of Non-pharmaceutical*

Grade & Compounded Pharmaceutical Grade Substances must be followed when using this agent. Typical doses providing up to 24 hours anesthetic duration are 2 g/kg IP in mice and 0.8-1.2 g/kg IP in rats.

APPENDIX II

Anesthetic Techniques and Recommended Agents for Rodents

Preanesthetics

Parasympatholytic (anti-cholinergic) agents such as atropine sulfate or glycopyrrolate, while employed in many veterinary anesthetic regimens, are not used in all rodent surgical procedures as they decrease intestinal peristalsis, resulting in ileus or gastrointestinal tract stasis. However, these agents should be incorporated into general anesthetic regimens for species such as guinea pigs and rats unless their use is expected to interfere with research results. These agents reversibly suppress the parasympathetic nervous system and are utilized to decrease respiratory tract secretions and inhibit the effects of vagal stimulation (e.g., bradycardia). The use of a parasympatholytic agent is imperative when manipulating the eye in all species to avoid complications that may result from stimulating the oculocardiac reflex resulting in bradycardia, arrhythmias and even death.

Tranquilizers or *sedatives* are frequently used as pre-anesthetics for general anesthesia. Animals premedicated with sedatives and/or tranquilizers are more manageable, are physiologically more stable, and require lower dosages of general anesthetics. Acepromazine and diazepam are examples of commonly used tranquilizers.

General Anesthesia

a. Injectable anesthetics – See Tables I & II for drug dosages

The rate of absorption of injectable anesthetic agents is directly related to the route of administration. In general, the relative rates of absorption for injectable anesthetic agents are as follows: Intravascular (IV) ≥ Intraperitoneal (IP) > Intramuscular (IM) > Subcutaneous (SC). Therefore, rates of onset and/or recovery from the same drug(s) may be markedly different if given by different routes. It is important to note that duration of anesthesia will vary with the specific anesthetic or anesthetic combinations used, their routes of administration, the animal's physiologic status, and the amount of stimulation during the procedure. For example, ketamine/xylazine given IP, versus IM, has a more rapid onset/recovery, and may require more frequent supplementation. Redosing of ketamine/xylazine or ketamine alone is only recommended if the surgical anesthetic plane cannot be maintained, i.e., return of the pedal or tailpinch reflex or there is a response to surgical stimulation.[28] See **Table I** for redosing instructions for ketamine/xylazine cocktail. Ketamine/alpha-2 adrenoceptor agonist combinations and pentobarbital are examples of commercially available injectable agents commonly used for general anesthesia. Tables I & II provide drug doses for various small laboratory animal species. Animals must always be weighed and agents administered on a per weight basis, as small differences in body weight may reflect a large difference in the amount of drug administered. Due to the small body weight of rodents, medications often require dilution prior to administration. Drug volumes are also guided by the animal's size. Please refer to Appendix IV for dilution instructions. Pleaserefer to Table III for additional guidance regarding appropriate administration volumes and routes

of administration, as well as available reversal agents. Drug dosages should **not** be extrapolated between different species, as there may be considerable differences in anesthetic response.

b. Inhalational anesthetics

Inhalational anesthetics produce a vapor that has anesthetic properties and thus are administered via the respiratory system. Their major benefit is that they typically allow for a quick and smooth recovery as they are rapidly cleared from the respiratory tract. Commonly used inhalational anesthetics include isoflurane and less commonly, sevoflurane. They are volatile, thus precautions must be taken to protect personnel and other animals from exposure to these agents. For this reason, isoflurane and sevoflurane must be used with a precision vaporizer to obtain working concentrations of 1-4%. Anesthesia machines regulate the flow of oxygen and the concentration of anesthetic gases. The anesthetic mixture may be delivered via an endotracheal tube, a tight-fitting facemask, or directly into a closed induction chamber depending on the species being anesthetized. Although difficult when using mice and rats, endotracheal intubation and specialized equipment is often necessary when positive pressure ventilation is required, e.g., survival thoracotomy. Rodents and other small laboratory animals may be anesthetized utilizing an "Anesthesia Box/Induction Chamber" which is connected to an inhalant vaporizer and a scavenging system.

Inhalational anesthesia can be used alone to induce and maintain anesthesia, or in conjunction with injectable anesthetic agents if supplemental anesthesia is necessary. When using a precision vaporizer and induction chamber, animals should always be placed in a clean, well-sealedinduction chamber prior to the instillation of inhalant anesthetic and oxygen; do not "pre-fill" thechamber with inhalant anesthetic. Excessive concentrations of isoflurane during induction may benoxious, causing irritation of the respiratory tract or even death. Once the animal is unable to move,or is laterally recumbent with a constant respiratory rate, the animal should be removed from the induction chamber. A nose cone connected to the anesthesia machine is then placed to form a tight-fitting seal around the nose, ensuring that waste anesthetic gases (WAGs) are contained and the animal is well-ventilated. Remember to switch the stop cocks to the "off" position (perpendicular to tubing) to the induction chamber and into the "on" position (parallel to tubing) to the nose cone.

When working with animals that have been exposed to a biological hazard, anesthesia must be administered in a Biosafety Cabinet (BSC). In this instance, the entire anesthetic gas delivery system must be placed into the BSC. When used in a BSC, medical air, rather than oxygen, should be used as the preferred delivery gas as the use of oxygen poses a fire risk and requires additional precautions including:

- Removing unnecessary combustible material from the cabinet;
- Move remaining combustible material (such as paper and cloth items; animal bedding) as far as possible from the flow of pure oxygen. Please note that plastic tubing is combustible;
- No flammable liquids should be inside the cabinet while pure oxygen is administered.
- Remove unnecessary sources of ignition;
- When electrosurgical instruments are used, oxygen flow should be temporarily stopped; and,

- In the event of igniting combustible material, the operator should be capable of immediately stopping oxygen flow and have a pre-determined method available to smother any burning material.

Chronic, low-level exposure to WAGs in the laboratory animal setting has been linked to an increased incidence of hepatic and renal toxicity, neurologic and reproductive dysfunction, and cancers in humans. Even though there has been significant improvement in decreasing WAG exposure, the use of activated charcoal canisters when anesthetizing rodents does not completely remove WAGs. Inhalational anesthetics should only be used with scavenging systems or within a chemical fume hood to ensure personnel safety. A BSC, unless ducted into the building's exhaust system, offers no scavenging of WAGs, therefore, a scavenging system is still required.

WAG exposure can be decreased substantially with appropriate gas-scavenging practices such as using passive scavenging activated charcoal canisters or an active scavenger system, such as a vacuum. Other possibilities include the use of local exhaust ventilation (e.g., snorkel device) or the use of an active gas scavenging systems. Additionally, low flow anesthetic regimens and optimal delivery system configurations, such as a tightly fitting nose cone, can help decrease personnel exposure.[41, 42]

Carbon dioxide from a commercially supplied cylinder or tank is an approved anesthetic in rodents for painful procedures of extremely short duration (30-60 seconds), e.g., retro-orbital bleeding techniques. Seventy percent or greater CO_2 concentrations should be utilized in a bell jar or CO_2 chamber. The use of CO_2 as the sole anesthetic agent is not permitted for invasive survival surgical procedures.

c. Hypothermia

Hypothermia is a safe and effective method to anesthetize neonatal altricial rodents[1] up to seven days old. Hypothermia will provide immobilization as well as anesthesia once the body temperature has decreased to 10-20ºC (50-68ºF). Nerve conduction is slowed or blocked during hypothermia thus minimizing pain and distress. An ice-water bath or crushed ice can be used to anesthetize rodent pups; however, the pup's skin must not come in direct contact with the ice or the water to avoid frostbite. This can be achieved by placing a glove between the pup and the ice, or alternatively, by placing a paper-lined test tube or a thin-walled plastic container between the pup and the ice. Anesthesia is achieved once the rodent pup's body temperature decreases to 10-20ºC (50-68ºF) and cardiac and respiratory functions have decreased significantly. Pups can be removed from the ice after 3-4 minutes or may be kept on a cooling platform over ice to provide a constant level of hypothermia for procedures up to 15 minutes. Pups should be recovered by warming in a gloved hand, or by placing in a Petri dish or clean cage on a warm water circulating heating pad or other warming device that offers evenly distributed heat at a finite temperature. Pups should be confirmed to be pink, breathing, and capable of spontaneous movement and wiped clean of blood and antiseptics prior to returning to the dam.

[1] Altricial rodents: Rodents requiring adult care for warmth, nursing, etc. at birth. Examples include mice, rats, and hamsters.

d. Local Anesthesia – See Tables I & II for drug doses

Local anesthetics interfere with the electrical conductance of nerve axons, thereby inhibiting afferent (sensory) impulses producing desensitization and analgesia. By preventing the influx of Na^+ ions, local anesthetics prevent depolarization and therefore prevent the conduction of electrical impulses. Local anesthetics may be used as an adjunct to general anesthetics when post-procedural pain or distress is expected to result from the surgical manipulation. For example, application of a local anesthetic agent to a peripheral nerve (sciatic) prior to ligation or transection in rodents has been shown to decrease the incidence of post-procedural autotomy (self-mutilation). Additionally, infiltration of a small volume (0.1-0.2 ml) of local anesthetic into the skin prior to making an incision has been shown to decrease afferent impulses from damaged nerves or tissue, thereby diminishing the neurochemical changes that can occur in the central nervous system which result in postoperative pain. It is important to note however, that a local anesthetic agent may not be required if the duration of pain of injection of the agent is as great as that produced by the procedure itself. Examples of injectable local anesthetic agents include lidocaine and bupivacaine.

Table I: Injectable Anesthetic Agents in Mice and Rats

Drug	Mice	Rats	Onset/Duration of Action
Alpha Chloralose[1,2]	85 mg/kg IP	45-55 mg/kg IP	Chemical restraint only. Discuss use with RARC Veterinarian
Atipamezole[3]	1 mg/kg IP		Return to righting reflex occurs within an average of 10 minutes in mice anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) IP if given 15 minutes after induction.
Atropine (anticholinergic)	0.02-0.05 mg/kg SC, IM, IV		
Bupivacaine[4]	0.1 ml (0.25% solution) SC, local infusion, topical	0.1-0.2 ml (0.25%-0.5% solution) SC, local infusion, topical	Onset: 5-10 min Duration: 1-2 hours
Chloral hydrate[1,2]	60-400 mg/kg IP	200-400 mg/kg IP	Duration: 60-90 min Discuss use with RARC Veterinarian

[1] Hazardous chemical and non-pharmaceutical grade compound, requires scientific justification for use
[2] Non-survival procedures only
[3] Atipamezole (Antisedan®) is package at 5 mg/ml. It will reverse dexmedetomidine in rats and mice at a dose of 0.1 to 1.0 mg/kg SC or IP
[4] Convulsions or death induced by the administration of local anesthetic occur at the following concentrations in mice: Bupivacaine >35 mg/kg, Lidocaine >110 mg/kg.

Table I: Injectable Anesthetic Agents in Mice and Rats (continued)

Drug	Mice	Rats	Onset/Duration of Action
Glycopyrrolate (anticholinergic)	0.01-0.02 mg/kg SC, IM		
Ketamine+ Xylazine (for surgical procedures)[5]	150 mg/kg IP 15 mg/kg IP	80-90 mg/kg IP 4-8 mg/kg IP	Onset: 2 min (mouse) Duration: 20-30 min (mouse), 20-40 min (rat) Recovery: 30-40 min (mouse)
Ketamine+Xylazine (for minor procedures or imaging)	100 mg/kg IP 10 mg/kg IP	40-80 mg/kg IP 5-10 mg/kg IP	Onset: 2 min (mouse) Duration: 20-30 min (mouse), 20-40 min (rat) Recovery: 30-40 min (mouse)
Ketamine+Xylazine+ Acepromazine	100 mg/kg IP 20 mg/kg IP 3 mg/kg IP	40 mg/kg IM 8 mg/kg IM 4 mg/kg IM	Onset: 2 min (mouse) Duration: 30-40 min (mouse),20-30 min (rat)
Ketamine + Dexmedetomidine[6]	50 mg/kg IP 0.5 mg/kg IP	60 mg/kg IP 0.2 mg/kg IP	Duration: 20-30 min (mouse),20-30 min (rat)
Lidocaine[4]	17.5 mg/kg SC, local infusion, topical (2% solution or 20 mg/ml)		Onset: 2-5 min Duration: up to 2 hours
Sodium Pentobarbital	50-90 mg/kg IP	40-60 mg/kg IP	Duration: 2-40 min (mouse), 20-60 min (rat)
Tribromoethanol (TBE, Avertin)[1]	400 mg/kg IP	N/A	Onset: 4 min Duration: 20 min Discuss use with RARC Veterinarian
Urethane[1,2]	2 g/kg IP	0.8-1.2 g/kg IP	Duration: Up to 24 hours Discuss use with RARC Veterinarian

[1] Hazardous chemical and non-pharmaceutical grade compound, requires scientific justification for use

[2] Non-survival procedures only

[3] Atipamezole (Antisedan®) is package at 5 mg/ml. It will reverse dexmedetomidine in rats and mice at adose of 0.1 to 1.0 mg/kg SC or IP.

[4] Convulsions or death induced by the administration of local anesthetic occur at the followingconcentrations in mice: Bupivacaine >35 mg/kg, Lidocaine >110 mg/kg.

[5] Redosing with ketamine/xylazine while an animal is still in surgical plane of anesthesia may result in mortality. The following redosing regimens reliably returns animals to a surgical plane for approximately 30-60 minutes: 50%-100% of the original ketamine dose or 25-50% of the original ketamine/xylazine dose. Inhalant anesthesia may also be used to prolong duration of anesthesia.

[6] Dexmedetomidine (Dexdomitor®) concentration is 0.5 mg/ml.

Table II: Injectable Anesthetic Agents in Hamsters and Guinea Pigs

Drug	Hamsters	Guinea Pigs	Duration of Action
Ketamine +Xylazine	100-200 mg/kg IP 7-10 mg/kg IP	30-50 mg/kg IM, IP 5 mg/kg IM, IP	Hamster: 30-60 min Guinea Pig: 30 min
Lidocaine	Not to exceed 4 mg/kg		< 1 hour-2 hours
Bupivacaine (0.25% - 0.5% solution)	Not to exceed 2 mg/kg		1-2 hours

Table III: Volumes (ml) and Routes of Administration by Species and Site

Species	Oral	SC	IP	IM	IV (bolus)	IV (infusion)
Mouse	0.25 (max 1.0)	Scruff 2-3 (<20G)	2-3 (<21G)	Quadriceps/ caudal thigh 0.05 (<23G)	Lateral Tail Vein 0.2 (<25G)	Max 25 ml/kg
Rat	3-5 (max 15)	Scruff/back 5- 10 (<20G)	5-10 (<21G)	Quadriceps/ caudal thigh 0.1 (<21G)	Lateral Tail Vein 0.5 (<23G)	Max 20 ml/kg
Hamster	0.5 (max 1.0)	Scruff 3-4 (<20G)	3-4 (<21G)	Quadriceps/ caudal thigh 0.1 (<21G)	Saphenous, cephalic, lingual 0.3 (25- 27G)	Max 25 ml/kg
GuineaPig	5-10 (max 35)	Scruff/back 5- 10 (<20G)	10-15 (<21G)	Quadriceps/ caudal thigh 0.3 (<21G)	Ear or saphenous Vein 0.5 (<23G)	Max 20 ml/kg

* All values are in milliliters per site, G = gauge of needle recommended

APPENDIX III

Analgesic Techniques and Recommended Agents for Rodents

Analgesic Administration (See Tables IV-V)

General considerations:

a. Preemptive analgesia is strongly recommended for all procedures that have the potential to cause pain. Preemptive analgesia involves administering the analgesic treatment PRIOR to surgery and surgical manipulations.
b. Local anesthetic techniques may be extremely beneficial for animals undergoing surgical procedures. Infiltration of a small volume of a long-acting local anesthetic, e.g., bupivacaine, prior to skin incision, or application to the wound edges before wound closure, will provide analgesia (1-2 hours) at the incision site, and is not associated with sedative effects observed with various systemic analgesics. Animals subjected to procedures that are expected to produce minimal to mild pain will typically benefit from a preemptive local anesthetic or preemptive NSAID or opioid.
c. Regardless of analgesic protocol, the animal should be monitored for signs of pain at least once daily for at least 72 hours following the procedure. Documentation of monitoring should be made on the blue Surgery Card. If signs of pain are observed, additional analgesics should be provided. For more details about post-operative care and documentation, please review the *Guidelines for conducting Survival Surgical Procedures in Rodents*.
d. Given the highly subjective nature of pain evaluation in rodents and other small laboratory mammals, PRN or as needed dosing regimens are NOT recommended. Rather, adhere to established dosing regimens.
e. Analgesic recommendations for common procedures and their associated pain category are provided in the table below

Commonly Performed Technical and Surgical Techniques and Recommended Analgesia[1]

Procedure	Pain level	Pre-emptive analgesic recommendations	Post-procedural analgesic recommendations
Small skin incision, catheter implantation, tail biopsy >28 days	Minimal to mild	Incisional anesthetic infiltration[2] **or** single dose of injectable analgesic[3]	

[1] Recommended analgesic protocols for commonly performed procedures. Age, health status, as well as prior procedures should be considered.
[2] Incisional anesthetic infiltration refers to a local anesthetic such as bupivacaine.
[3] Injectable analgesic refers to an opioid such as buprenorphine or NSAID such as meloxicam.

Commonly Performed Technical and Surgical Techniques and Recommended Analgesia[1] (continued)

Procedure	Pain level	Pre-emptive analgesic recommendations	Post-procedural analgesic recommendations
Craniotomy in adult animals, subcutaneous pump implantation	Mild	Incisional anesthetic infiltration[2] **and** single dose of injectable analgesic[3]	
Soft tissue removal, subcutaneous tumor resection	Mild to moderate	Incisional anesthetic infiltration[2] **and** injectable (NSAID **or** opioid) analgesic	Injectable (NSAID **or** opioid) analgesic for a minimum of 24 hrs.[1]
Laparotomy, muscle transection	Moderate	Incisional anesthetic infiltration[2] **and** injectable (NSAID **and** opioid) analgesic[3]	Injectable (NSAID **or** opioid) analgesic for a minimum of 48 hrs.[4]
Thoracotomy, extensive soft tissue manipulation, orthopedic procedure, limb amputation, nerve biopsy, burns, induced ischemia	Moderate to severe	Incisional anesthetic infiltration[2] **and** injectable (NSAID **and** opioid) analgesic[3]	Injectable (NSAID **and** opioid) analgesic for a minimum of 72 hrs.[4,5]

[1]Recommended analgesic protocols for commonly performed procedures. Age, health status, as well as prior procedures should be considered.
[2] Incisional anesthetic infiltration refers to a local anesthetic such as bupivacaine.
[3]Injectable analgesic refers to an opioid such as buprenorphine or NSAID such as meloxicam.
[4] If Ethiqa XR® is given pre-emptively, no additional post-operative opioids are required.
[5] If Ethiqa XR® is given pre-emptively, no additional post-operative opioids are required for mice or rats when used in combination with an NSAID.

Table IV: Analgesic Agents for Mice and Rats

Non-steroidal Anti-inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs are compounds that are not steroidal and suppress inflammation, which can result in pain. The NSAIDs have various mechanisms of action and vary in their ability to influence inflammation (see Appendix I for more details). NSAIDs are not controlled substances and are generally used for procedures expected to result in mild to moderate pain.

Drug	Mice	Rats
Carprofen	2-5 mg/kg SC q12-24 hrs	2-5 mg/kg SC q24 hrs
Meloxicam	1-2 mg/kg PO, SC q24 hrs	1-2 mg/kg PO, SC q24hrs
Ketoprofen	2-5 mg/kg SC q24 hrs	2-5 mg/kg SC q24 hrs

Opioids

All opioids, including buprenorphine, are controlled substances. The formulation of buprenorphine used, and the frequency of administration should depend on the expected pain level of the procedure performed and the clinical appearance of the animal. Buprenorphine should only be given SC (not IP or IM) and is not recommended for use in mice or rats with pre-existing respiratory deficiencies.

Drug	Mice[1]	Rats[2]
Buprenex® (0.3 mg/ml)	0.5-1.0 mg/kg SC q4 to 12 hrs	0.02-0.05 mg/kg SC q6 to 12 hrs
Ethiqa XR® (1.3 mg/ml)[3,4]	3.25 mg/kg SC once for 48 hours of action[5,6]	0.65 mg/kg SC once for 72 hours of action[6]

[1]Buprenorphine when given in high doses has been known to elicit hyperactivity in mice.
[2]Buprenorphine when administered multiple times to rats has at times been associated with pica (rat ingests bedding or paper products provided within the cage).
[3]Shake before each use to ensure uniform suspension.
[4]Avoid direct contact with human skin or mucus membranes.
[5]Existing data have shown that a single SC dose of Ethiqa XR provides only 48 hours of pain control in mice, therefore, institutional guidelines differ from the manufacturer's dosing recommendations for mice.
[6]If needed, a single repeat dose may be given 48 hours (mice) or 72 hours (rats) after the initial dose.

Table V: Analgesic Agents for Hamsters and Guinea Pigs

Drug	Hamsters	Guinea Pigs
Ketoprofen	5 mg/kg SC q24 hrs	1 mg/kg SC q12-24 hrs
Carprofen[1]	5 mg/kg q24 hrs	2-4 mg/kg SC q12-24 hrs
Meloxicam [1, 2]	1-2 mg/kg PO, SC q24 hrs	0.1-0.3 mg/kg PO, SC q24 hrs
Buprenorphine [1,2,3]	0.25-0.5 mg/kg SC q6-8 hrs	0.05 mg/kg SC q8-12 hrs
Morphine [2,3]	2-5 mg/kg SC q2-4 hrs	10 mg/kg SC q2-4 hrs

[1]For control of mild to moderate levels of postoperative pain.
[2]Combining an opiate (e.g., buprenorphine) and an NSAID (e.g., meloxicam or carprofen) provides the most effective analgesic plan especially for moderate to severe postoperative pain.
[3]For control of moderate to severe postoperative pain.

Note: There may be considerable individual and strain variation to administration of the same analgesic agent. It is therefore essential to assess analgesic effect in each individual animal.

APPENDIX IV

Dilution of Commonly Used Anesthetics and Analgesics

Many drugs are only available in concentrations, which when administered to rodents, would be exceeding small and difficult to administer accurately. Therefore, they often require dilution of the drug with sterile saline or sterile water to ensure accurate dosing. These guidelines are providedto help prepare appropriate dilutions of common anesthetic cocktails and analgesics.

Sterile procedures and containers must be used when preparing dilutions. Dilutions must not be stored in screw top containers or syringes, only sterile containers with an injection port must be used. Veterinary Services (VS) can provide sterile injection vials or ordering information. Controlled substances (CS), including dilutions and cocktails containing at least one CS, must remain locked in an approved double locked box. Please review the EHS guidance document *Security of DEA Controlled Substances* (WCM), or the *Guidelines for Requesting, Using and Storing Controlled Substances* (Burke), or the *Policy on Controlled Substances in Laboratory and Animal Research* (MSK). for more information.

A Controlled Substance Usage Log for Dilution/Cocktail (CSUL) must be created for each new cocktail created. Dilutions must be labeled with the drug, concentration, date the dilution was made, and a "use by" date **1 month from the date of dilution** except for dilutions of ketamine/xylazine. Ketamine/xylazine cocktails must be labeled with the name and volumes of each drug, the date of dilution and the expiration date, which is **6 months** from the date of mixing the cocktail or the date of ketamine or xylazine expiration, whichever comes first. At WCM/HSS, unused mixed solutions of controlled substances must be handled as described in the EHS guidance document *Security of DEA Controlled Substances* (WCM). At Burke, unused mixed solutions of controlled substances must be returned to Burke Drug Ordering Services along with the completed CSUL as described in the *Guidelines for Requesting, Using and Storing Controlled Substances* (Burke). At MSK, solutions should be handled as described in the MSK Policy on Controlled Substances in Laboratory and Animal Research.

Anesthetic Dilution & Dosing Recommendations

To accomplish accurate dosing and avoid the adverse effects of overdosing or requiring additional doses of anesthetic, all animals should be weighed prior to being placed under anesthesia. For accurate dosing, it is recommended that you use the smallest syringe available for dosing. For volumes of 0.1 ml or less, an insulin syringe should be used. For volumes
of 1 ml or less, a tuberculin syringe should be used. Syringes purchased with the needle attached are known as "low volume" syringes and are preferred over syringes with detachable needles to decrease the amount of dead space in the syringe. This allows for more accurate dosingand less loss of drug in the hub of the syringe. However, more viscous drugs (e.g., Ethiqa XR®, Fatal Plus®) may require a larger needle size.

Below are directions for making ketamine/xylazine cocktails for restraint and surgical procedures in mice and rats. Doses are provided for reference and volumes to be administered are provided on a per 10g (mouse) or 100g (rat) basis as well as aper gram basis.

Ketamine/Xylazine Cocktail Preparation for Mice

Drug	**Drug stock concentration (mg/ml)**	**Volume used for cocktail**	**Ketamine/xylazine concentration**
Ketamine	100 mg/ml	1.0 ml	
Xylazine	100 mg/ml	0.1 ml	
Saline	Sterile, isotonic	8.9 ml	
Combination Cocktail		**10 ml total**	**10 mg/ml ketamine and 1 mg/ml xylazine**

Sample Dosing of Ketamine/Xylazine for Mice by Weight

Mouse weight in grams	Recommended volume of cocktail for **restraint** in ml (0.01 ml/ gram body weight)	Recommended volumes for redosing for restraint	Recommended volume of cocktail for **surgery** in ml (0.015 ml/gram body weight)	Recommended volumes for redosing for surgery
10	0.1	25-50% of original dose	0.15	25-50% original dose
20	0.2	25-50% of original dose	0.3	25-50% original dose
30	0.3	25-50% of original dose	0.45	25-50% original dose

Ketamine/Xylazine Cocktail for Restraint and Surgical Procedures in Rats

Drug	**Drug stock concentration (mg/ml)**	**Volume used for cocktail**	**Ketamine/Xylazine Concentration**
Ketamine	100 mg/ml	2.0 ml	
Xylazine	100 mg/ml	0.2 ml	
Saline	Sterile, isotonic	7.8 ml	
Combination Cocktail		**10 ml total**	**20 mg/ml ketamine and 2 mg/ml xylazine**

Sample Dosing of Ketamine/Xylazine for Rats by Weight

Rat weight ingrams	Recommended volume of cocktail for **restraint** in ml (0.003 ml/gram body weight)	Recommended volumes for redosing for restraint	Recommended volume of cocktail for **surgery** in ml (0.004 ml/gram body weight)	Recommended volumes for redosing for surgery
200	0.6	25-50% original dose	0.8	25-50% original dose
300	0.9	25-50% original dose	1.2	25-50% original dose
350	1.05	25-50% original dose	1.4	25-50% original dose
400	1.2	25-50% original dose	1.6	25-50% original dose

Pentobarbital

The recommended anesthetic dose for a mouse is 50-90 mg/kg IP and the recommended dose for a rat is 40-60mg/kg IP. If you wish to use pentobarbital for a procedure that requires the animal to be anesthetized for a procedure or survival or non-survival surgery, Nembutal® must be used. Nembutal® is supplied as 50 mg/ml sodium pentobarbital solution which should be diluted to a concentration of 5 mg/ml with sterile water or sterile saline for use as an anesthetic agent in mice (use in rats does not requiredilution). To achieve a 5 mg/ml solution, add 1.0 ml of pentobarbital (50 mg/ml) to 9 ml of sterile water or sterile saline.

If you wish to use pentobarbital for a procedure which results in irreversible death of an animal, such as perfusion, exsanguination, or tissue harvest, a dilution of a commercial product intended for animal euthanasia may be used for mice and rats (not for other rodents). Fatal Plus ® is supplied as a 390 mg/ml solution of sodium pentobarbital solution. Please note that other brands of euthanasia solution (e.g., Euthasol®) may contain other additives and the potential impact of these additives on your research should be considered. To achieve a 19.5 mg/ml solution (mice), add 0.25 ml of Fatal Plus ® to 4.75 ml of sterile water or sterile saline. To achieve a 39 mg/ml solution (rats), add 0.5 ml of Fatal Plus ® to 4.5 ml of sterile water or sterile saline.

Analgesic Dilution & Dosing Recommendations for Mice and Rats

Carprofen

The recommended mouse and rat carprofen dose is 2-5 mg/kg every 12-24 hours (Please see tables above for further details). Carprofen is available from the supplier in a 50 mg/ml solution.

Mouse: Add 0.1 ml of carprofen (50 mg/ml) to 4.9 ml of sterile water for injection. This results in a 1 mg/ml solution. Using a dose of 5 mg/kg, an adult mouse would require 0.05 ml per 10g of bodyweight as a SC injection.

Rat: Add 0.5 ml of carprofen (50 mg/ml) to 4.5 ml of sterile water from injection for a total volume of 5 ml. This will result in a 5 mg/ml solution. Using a dose of 5 mg/kg, an adult rat would require 0.1 ml per 100g of body weight as a SC injection.

Buprenorphine

Two different pharmaceutical-grade preparations of buprenorphine are available, with different dosages and durations of action.

Ethiqa XR® (1.3 mg/ml buprenorphine extended-release injectable suspension): The recommended dose is **3.25 mg/kg SC for mice** and **0.65 mg/kg SC for rats**. Each dose of Ethiqa XR® provides pain relief for at least 48 hours in mice and 72 hours in rats. Another dose should not be given until 48 hours (mice) or 72 hours (rats) after the last dose. **Ethiqa XR® should not be diluted or heated and use of a 20- to 23- gauge needle is recommended.** Vigorously shake the vial before withdrawing into a syringe to ensure a uniform suspension. Please refer to the *Ethiqa XR® User's Guide* (WCM/HSS, Burke, or MSK) for more details. It should be noted that adverse events, including death, have been reported when NSAIDs and Ethiqa XR have been administered concomitantly in mice. Therefore, only the doses recommended in this document should be used.

Buprenex® (0.3 mg/ml buprenorphine HCl):

Mouse: The recommended Buprenex® dose is 0.5-1 mg/kg SC every 4-12 hours. Mix one vial (1 mL) of 0.3 mg/ml of Buprenex® in 3 ml of sterile saline or sterile water for injection to achieve a 0.075 mg/ml solution. Using a dose of 0.5 mg/kg, an adult mouse would require 0.06-0.08 mL per 10g of body weight. A 4 ml diluted vial will provide approx. 20 doses. For a larger volume, mix two vials of Buprenex® with 6 ml of diluent.

Rat: The recommended Buprenex® dose is 0.02-0.05 mg/kg by SC injection every 6-12 hours.

Mix one vial (1.0 mL) of 0.3 mg/ml Buprenex® in 9 ml of sterile saline or sterile water for injection to achieve a 0.03 mg/ml dilution. Using a dose of 0.05 mg/kg, an adult rat would require 0.13 ml per 100g of body weight. Dilution may not be required for large (> 600g) rats.

Meloxicam

Mouse: The recommended meloxicam dose is 1-2 mg/kg SC or PO every 24 hours. When using injectable meloxicam, it is recommended that the 5 mg/ml stock concentration is diluted 10 times with sterile water or saline before administration by adding 1 ml (5 mg/1ml vial)
+ 9 ml sterile water = 0.5 mg/ml concentration. Using a dose of 2 mg/kg, an adult mouse would require 0.04 ml per 10g of body weight.

Rat: Dilution of injectable meloxicam is not required for adult rats. The recommended meloxicam dose is 1-2 mg/kg SC or PO every 24 hours. Using a dose of 2 mg/kg, an adult rat would require 0.04 mL (undiluted) per 100g of body weight.

Bupivacaine:

This is a local anesthetic used to infiltrate surgical sites and provide pain relief for 4-8 hours.

Mouse: Recommended dose is 0.1 ml of a 0.25% solution. Use of a 0.5% solution in mice requires dilution. Starting with a 0.5% stock solution (5 mg/ml), add 1 ml of sterile saline to 1 ml of the 0.5% solution in a sterile vial. This produces a solution of 0.25% bupivacaine. Use 0.1 ml per mouse for SC infiltration along the incision line or topically into theincision prior to closure.

Rat: Recommended dose is 0.1-0.2 ml of either the 0.25% or 0.5% solution. Dilution of bupivacaine is not required for rats.

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