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	<b>Guidance Document</b>
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## **Veterinary Recommendations for Anesthesia and Analgesia**

This Guidance Document is to establish recommendations for anesthesia and analgesia for use in research animals.

### PRINCIPLES OF ANESTHESIA AND ANALGESIA

1. **The proper anesthetic and analgesic agents must be used in order to eliminate or reduce the potential for pain and distress during the peri-operative period.**
2. Withholding anesthesia or analgesia must be justified and approved in the IACUC protocol.
3. A multimodal approach to analgesia should be employed to offer the best broad-spectrum pain control possible. This includes the use of different categories of analgesics in combination to address different sources of pain perception/stimulation.

For example, a surgical procedure may use a local anesthetic block of lidocaine and bupivacaine at the incision site, and systemic administration of an NSAID for inflammatory pain and an opioid.

4. According to the 8<sup>th</sup> edition of the Guide for the Care and Use of Laboratory Animals (NRC), "Guidelines for the selection and proper use of analgesic and anesthetic drugs should be developed and periodically reviewed and updated as standards and techniques are refined."

### ANESTHESICS

1. Inhalant Anesthetics
  - a. The inhalant anesthetics include gases such as isoflurane and sevoflurane. These anesthetics require an anesthetic machine set-up. In addition, use of a scavenger system is required to prevent personnel exposure to the waste anesthetics. For short procedures it may be possible to administer inhalant anesthesia via a drop jar. For this procedure a cotton ball or gauze soaked with the anesthetic is placed in a jar with the animal. This procedure must be performed under a fume hood, the animal CANNOT contact anesthetic, and can only be done for minor quick procedures. Animals will recover quickly after removal from jar.
  - b. Advantages: safe and reliable, predictable and rapid control of anesthetic depth, not controlled substances
  - c. Disadvantages: induction must be closely monitored, personal training, special equipment required, potential risk to staff (if not appropriately scavenged)
2. Injectable anesthetics
  - a. Injectable general anesthetics include ketamine/xylazine and pentobarbital. Most of the commonly used agents are administered via intraperitoneal (IP) injection (figure 4).
  - b. Local anesthetics are often delivered subcutaneously along the incision site. They could also be used in nerve blocks or epidural administration. Local anesthetics are not adequate as the only analgesic for any surgical procedure unless scientifically justified in the protocol.

- c. Advantages: They can be used without expensive supporting equipment such as the anesthesia machines required with the use of inhalants, they are easily transported, and are relatively inexpensive.
- d. Disadvantages: Prolonged recovery times. The animal will have to metabolize the drug in order to completely recover from anesthesia. In addition, once the agent is injected, the anesthetic depth cannot be adjusted throughout the procedure except to achieve a deeper anesthetic plane by giving additional drug if the animal demonstrates signs of arousal. Animals which are sick or compromised may have a difficult time with these anesthetics due to changes in their ability to metabolize the drugs. Also, many of the commonly used injectable anesthetics are controlled substances which will require the laboratory comply with all rules regarding controlled substances and obtain a DEA license.

### 3. Monitoring Anesthetic Depth

Anesthetic depth should be gauged prior to conducting any surgical manipulation and throughout the surgical procedure. Loss of reflexes (e.g., pedal, corneal, palpebral) can be used to assess appropriate anesthetic plane. There should not response to toe pinch. Signs of Inadequate anesthetic depth include purposeful movement, reflexes present, response to painful stimulus, or twitching whiskers. Equipment (e.g. pulse oximeter) may also be used to monitor depth of anesthesia. Changes in heart rate, respiratory rate, or blood pressure may indicate whether an animal is at too light or too deep of an anesthetic plane. Depth of anesthesia should be assessed every 10-15 minutes during surgery.

## ANALGESIA

### 1. Pain assessment and Analgesia

Assessing pain is difficult in animals. Some animals are species of prey and are adapted to hide signs of pain and distress. Clinical signs associated with pain are species specific, but some common signs of all species include changes in appearance such as hunched, scruffy, porphyrin staining (rats/mice), or changes in activity, including less active or inactive, hyperactive or pacing, abnormal postures such as back arching, belly pressing, wound guarding, or writhing. Also, decreased appetite, isolation from cage mates, exaggerated or decreased response to handling, vocalization can be used to gauge pain and distress. Recently, there has been some focus on assessing rodent facial expression or grimace scale in order to assess pain (Figure 1). It is important to pay close attention to the animal's appearance and behavior post-surgery in order to observe subtle changes that may indicate the need for additional pain management.

Analgesia should be administered to **ALL** post-surgical animals unless otherwise justified in the protocol. If there is concern regarding an animal's clinical condition post-surgery and additional analgesia is necessary, contact DLAR veterinary staff for further guidance on treating the animal.

Whenever possible multimodal analgesia is recommended. This involves providing a more 'balanced analgesia' through multiple methods or modalities. Local anesthetics at the incision site are often used in conjunction with a stronger opioid or NSAID analgesic.

## RECOMMENDATIONS BY PROCEDURE

See specific doses, routes, and frequencies in the formularies below.

### 1. Rodent laparotomy (example major surgery)

Anesthesia induction via isoflurane in an induction chamber at 3-5% followed by isoflurane via facemask at 2-4%. Buprenorphine or other moderate to strong analgesic is given immediately after induction prior to patient prep to allow adequate time to reach therapeutic levels. Subsequently, moderate to strong analgesia is administered at appropriate intervals for 48 hours minimum post-operatively. Rodents are monitored closely thereafter to evaluate for signs of pain, and additional analgesia is given until evidence of pain is no longer present.

2. Rodent subcutaneous implant or vascular catheter placement (example minor surgery)

Anesthesia induction via ketamine-xylazine. Carprofen is given immediately after induction prior to patient prep to allow adequate time to reach therapeutic levels. Carprofen is given as directed for 24 hours minimum post-operatively. Rodents are monitored closely thereafter to evaluate for signs of pain, and additional analgesia is given until evidence of pain is no longer present.

3. Swine laparotomy

Anesthesia induction via ketamine-acepromazine. Pre-emptive analgesia is given immediately after induction prior to patient prep to allow adequate time to reach therapeutic levels; buprenorphine (for moderate pain; time to effect - 30 minutes IV & 60 minutes IM) and NSAID (time to effect - 60 minutes IV or IM). Following endotracheal intubation, anesthesia is maintained with isoflurane. Local anesthetics, lidocaine and bupivacaine, are given SC at the location of planned incision. Post-operative analgesia, buprenorphine and NSAID, is given at appropriate intervals for at least 48 hours postoperatively. Swine are monitored closely postoperatively to evaluate for signs of pain, and additional analgesia is given until evidence of pain is no longer present.

4. Dog thoracotomy

Anesthesia induction via ketamine-diazepam. Pre-emptive analgesia is given immediately after induction prior to patient prep to allow adequate time to reach therapeutic levels; buprenorphine (for severe pain; time to effect - 30 minutes IV & 60 minutes IM) and NSAID (time to effect - 60 minutes IV or IM). A fentanyl transdermal patch is secured on skin (time to effect - 12-24 hours). Following endotracheal intubation, anesthesia is maintained with isoflurane. Local anesthetics, lidocaine and bupivacaine, are given SC at the location of planned incision. Once skin incision is made, an intercostal nerve block is performed. Post-operative analgesia is given at appropriate intervals for at least 72 hours postoperatively, buprenorphine for 12-24 hours until fentanyl takes effect, fentanyl transdermal patch in place for 72 hours, and NSAID for 3-5 days. Dogs are monitored closely postoperatively to evaluate for signs of pain, and additional analgesia is given until evidence of pain is no longer present.

## FORMULARY FOR MOST COMMONLY USED SPECIES AND DRUGS

Mouse and Rat Formulary					
Analgesics	Dose (mg/kg)		Route	Freq	Comments
	Mouse	Rat			
Acetaminophen Oral dose		100-300	PO	q4h	Not adequate as a sole analgesic except for very minor pain. May be combined with another class of analgesic for post-op pain.
Water bottle	1-2 mg/ml drinking water	6 mg/ml drinking water	PO		Change water every other day. Water must be placed on cage 48 hours prior to painful procedure in order for rats to acclimate.
<b>Recommended:</b> Buprenorphine	0.05 – 0.1	0.01 – 0.05	SC	q12h	Excellent for moderate to severe pain.
Carprofen	5	5	SC	q24h	Good for mild to moderate pain.
Meloxicam	1-5	1-2	PO, SC	q24h	
Tramadol	20 - 40	5 - 20	IP		For chronic or severe pain. Literature does not define recommended dosing interval in rodents. In other species administered up to TID. Start at BID dosing.
Anesthetics	Mouse	Rat	Route	Duration	Comments
<b>Recommended:</b> Isoflurane	2-5%	2-5%	Inhalation		Gold standard anesthetic. Must have appropriate equipment to use safely (precision vaporizer and scavenging).
Ketamine / xylazine	90-120 10	40-80 5-10	IP	30-45 min	Ketamine combinations are the next best anesthetic if isoflurane cannot be used. <b>Do not re-dose xylazine</b> ; if additional dose needed provide ~1/3 dose ketamine
Ketamine / medetomidine	50-75 1-10	60 0.4	IP		
Ketamine / xylazine / acepromazine	100 2.5 2.5	40 8 4	IM, IP		
Pentobarbital	30-90	30-60	IP	60-120 min	Not readily available.
Tribromoethanol	250	Not recomm- ended	IP		Very short term anesthesia, not recommended for survival procedures; inappropriate storage or mixing may result in toxicity.
Reversal Agents	Mouse	Rat	Route	Duration	Comments
Yohimbine	0.2	0.2	IP	NA	Reverses xylazine
Atipamazole	1	1	SC	NA	Reverses xylazine and medetomidine
Local anesthetics	Mouse	Rat	Route	Duration	Comments

Bupivacaine / Lidocaine mixture	1.5 mg/kg	1.5 mg/kg	SC	4-8 hrs	Slow onset, long duration	Mix together in same syringe for infiltration around incision
	0.5 mg/kg	0.5 mg/kg		<1 hr	Rapid onset, short duration	

Dog Formulary					
Analgesics	Dose (mg/kg)	Route	Frequency	Comments	
<b>OPIOIDS</b>					
<b>Recommended:</b> Buprenorphine	0.01-0.03	IV/IM/SC	Q6h	Mild to moderate pain	
Fentanyl transdermal	<10kg 25 µg/h 10-25kg 50 µg/h >25kg 100 µg/h	dermal patch	q72h	Severe pain; 24 hours for maximal effect	
<b>NSAIDS/OTHER</b>					
<b>Recommended:</b> Carprofen	2.2 4.4	PO/IV/SC	Q12h Q24h	Excellent synergistic effect with opioids	
Meloxicam	0.2 mg/kg once, then 0.1 mg/kg	IV/SC/PO	q24h	Excellent synergistic effect with opioids	
Acetaminophen	10-15	PO	q6-8h	Antipyretic, mild pain	
<b>Anesthetics</b>					
<b>INHALATION</b>					
<b>Recommended:</b> Isoflurane	3-5% induction 1-3% maintenance	Inhalation	continuous	Must use precision isoflurane vaporizer	
Sevoflurane	5-7% induction 2-4% maintenance	Inhalation	continuous	Must use precision sevoflurane vaporizer	
<b>INJECTABLE</b>					
Ketamine / diazepam	5.0 mg/kg 0.25 mg/kg	IV in same syringe	As needed	Use for anesthetic induction; duration 20-30 minutes	
Ketamine / midazolam	5.0 mg/kg 0.25 mg/kg	IV in same syringe	As needed	Use for anesthetic induction; duration 20-30 minutes	
Propofol	5.5-8.0 mg/kg* = induction  0.4 mg/kg/min* = maintenance	IV  IV	Slowly to effect  Continuous rate infusion (CRI)	Must administer slowly over 30-60 seconds to avoid apnea, hypotension. Rapid recovery in 10-20 minutes	
Pentobarbital*	25-30 mg/kg	IV	Slowly to effect; single or intermittent bolus	Single dose duration approximately 45 minutes	

Local anesthetics				
Bupivacaine / Lidocaine mixture	1.5 mg/kg 0.5 mg/kg	SC	Once	Mix in same syringe for infiltration or local nerve block. Bupivacaine has slow onset (20-30 minutes), longer duration (4-8 hours); Lidocaine has rapid onset (5-10 minutes), shorter duration (1-2 hours).
Neuromuscular Blocking				
Pancuronium	0.05-0.1 mg/kg, then 0.01 mg/kg	IV	Once, then as needed	Duration of effect 30-45 minutes
Reversal agents				
Atropine Neostigmine methylsulfate	0.02 mg/kg 0.04 mg/kg	IV	Once No more than 3 times	Reverses neuromuscular block; give atropine first

Swine Formulary <sup>3</sup>				
Analgesics	Dose (mg/kg)	Route	Frequency	Comments
<b>OPIOIDS</b>				
<b>Recommended:</b> Buprenorphine	0.01-0.05	IV/IM/SC	q8-12h	Mild to moderate pain
Butorphanol	0.1-0.3	IM/SC	q4-6h	Mild to moderate pain
Fentanyl transdermal	5 µg/kg/h	dermal patch	q72h	Severe pain; dosage variable depending upon breed, age, patch location, heat, moisture, procedure
<b>NSAIDS</b>				
<b>Recommended:</b> Carprofen	2.0 3.0-4.0	PO/IV/SC/I M	q12-24h q24h	Excellent synergistic effect with opioids
Meloxicam	0.1 mg/kg 0.4 mg/kg	PO IV/SC	q24h	Excellent synergistic effect with opioids
<b>Anesthetics</b>				
<b>INHALATION</b>				
<b>Recommended:</b> Isoflurane	3-5% induction 1.5-3% maintenance	Inhalation	continuous	Must use precision isoflurane vaporizer
Sevoflurane	5-7% induction 3-4% maintenance	Inhalation	continuous	Must use precision sevoflurane vaporizer
<b>INJECTABLE</b>				
Ketamine/ acepromazine	22-33 mg/kg 1.1 mg/kg	IM/SC	once	Use for anesthetic induction; duration 30 minutes; slightly cardio-depressant

Ketamine/ midazolam	33.0 mg/kg 0.5 mg/kg	IM/SC	once	Use for anesthetic induction; duration 45-60 minutes; profoundly hypothermic due to peripheral vasodilation; terminal procedures only
Ketamine/ xylazine	20 mg/kg 2 mg/kg	IM/SC	Once	Use for anesthetic induction; use anticholinergic to reverse cardiodepression/heart block
Telazol® (tiletamine/zolazepam)	2.0-8.8 mg/kg	IM/SC	Once	20 minutes immobilization; use for minor surgery & anesthetic induction; hypothermia, cardiodepression
Telazol®/ xylazine	4.4 mg/kg 2.2 mg/kg	IM/SC	Once	Use for anesthetic induction and intubation; not recommended for cardiovascular studies
Propofol	0.83-1.66 mg/kg induction  14-20 mg/kg/min* maintenance	IV  IV	Slowly to effect  Continuous rate infusion (CRI)	Must administer slowly over 30-60 seconds to avoid apnea, hypotension. Effective sedation and muscle relaxation with minimal cardiodepression at lower dosages; poor analgesia at lower dosages
<b>Reversal agents</b>				
Yohimbine	0.05-0.1 mg/kg	IV slowly	Once	Reverses sedative, cardiodepression, analgesic effects of xylazine
<b>Local anesthetics</b>				
Bupivacaine / Lidocaine mixture	1.5 mg/kg 0.5 mg/kg	SC	Once	Mix in same syringe for infiltration or local nerve block. Bupivacaine has slow onset (20-30 minutes), longer duration (4-8 hours); Lidocaine has rapid onset (5-10 minutes), shorter duration (1-2 hours).
<b>Neuromuscular Blocking</b>				
Pancuronium	0.02-0.15 mg/kg	IV	Once, then as needed	Duration of effect 30-45 minutes
<b>Reversal agents</b>				
Atropine Neostigmine methylsulfate	0.02 mg/kg 0.04 mg/kg	IV	Once No more than 3 times	Reverses neuromuscular block; give atropine first

<b>Cat Formulary</b>				
<b>Analgesics</b>	Dose (mg/kg)	Route	Frequency	Comments
<b>OPIOIDS</b>				
<b>Recommended:</b> Buprenorphine	0.01-0.03	IV/IM/SC	q6h	Mild to moderate pain

		PO (sublingual)	q6-12h	
Fentanyl transdermal	25 µg/h	dermal patch	q72h	Severe pain; 6-12 hours for maximal effect
<b>NSAIDS</b>				
<b>Recommended:</b> Meloxicam	0.1 mg/kg IV/SC/PO once, then 0.05 mg/kg PO	IV/SC/PO	q24h	Excellent synergistic effect with opioids; chronic dosing PO only
<b>Anesthetics</b>				
<b>INHALATION</b>				
<b>Recommended:</b> Isoflurane	3-5% induction 1-3% maintenance	Inhalation	continuous	Must use precision isoflurane vaporizer
Sevoflurane	5-8% induction 2-4% maintenance	Inhalation	continuous	Must use precision sevoflurane vaporizer
<b>INJECTABLE</b>				
Ketamine/ diazepam	5.0 mg/kg 0.25 mg/kg	IV in same syringe	As needed	Use for anesthetic induction; duration 20-30 minutes
Ketamine/ midazolam	5-10 mg/kg 0.2-0.4 mg/kg	IM in same syringe	As needed	Use for anesthetic induction; duration 20-30 minutes
Ketamine/ xylazine	10 1	IM	Once	Administer xylazine first as stimulation of chemoreceptor trigger zone frequently causes vomiting; wait 10 minutes, then administer ketamine
<b>Reversal agents</b>				
Yohimbine	0.05-0.1 mg/kg	IV slowly	Once	Reverses sedative, cardiodepression, analgesic effects of xylazine
<b>Local anesthetics</b>				
Bupivacaine / Lidocaine mixture	1.5 mg/kg 0.5 mg/kg	SC	Once	Mix in same syringe for infiltration or local nerve block. Bupivacaine has slow onset (20-30 minutes), longer duration (4-8 hours); Lidocaine has rapid onset (5-10 minutes), shorter duration (1-2 hours).
<b>Neuromuscular Blocking</b>				
Pancuronium	0.05-0.1 mg/kg, then 0.01 mg/kg	IV	Once, then as needed	Duration of effect 30-45 minutes
<b>Reversal agents</b>				
Atropine Neostigmine methylsulfate	0.02 mg/kg 0.04 mg/kg	IV	Once No more than 3	Reverses neuromuscular block; give atropine first

			times	
Edrophonium	0.5 mg/kg	IV	Once No more than 5 times	Reverses neuromuscular block; complete reversal takes 5-45 minutes

<b>Rabbit Formulary</b>				
<b>Analgesics</b>	Dose (mg/kg)	Route	Frequency	Comments
<b>OPIOIDS</b>				
<b>Recommended:</b> Buprenorphine	0.01-0.05	IV/SC	q6-12h	Mild to moderate pain
Fentanyl transdermal	½ 25 µg/h patch for 3 kg rabbit <sup>1</sup>	dermal patch	q72h	Severe pain; do not cut patch, cover ½ not in use
<b>NSAIDS</b>				
<b>Recommended:</b> Meloxicam	0.3-0.6 mg/kg	SC/PO	q24h	Excellent synergistic effect with opioids
Carprofen	2-4 mg/kg 1.0-2.2 mg/kg <sup>1</sup>	SC PO	q24h q12h	Excellent synergistic effect with opioids
<b>Anesthetics</b>				
<b>INHALATION</b>				
<b>Recommended:</b> Isoflurane	3-5% induction 1-3% maintenance	Inhalation	continuous	Must use precision isoflurane vaporizer
<b>INJECTABLE</b>				
<b>Recommended:</b> Ketamine/ Xylazine	35-50 mg/kg 5-10 mg/kg	IM	If additional doses needed to prolong anesthesia provide 1/3 dose ketamine only	Minor surgery only; lower doses used for anesthetic induction when isoflurane used for anesthetic maintenance
Ketamine/ Medetomidine	15-25 mg/kg 0.25-0.5 mg/kg	SC/IM		Minor surgery only; used for anesthetic induction when isoflurane used for anesthetic maintenance
Ketamine/ Xylazine/ Buprenorphine	35 mg/kg 5 mg/kg 0.03 mg/kg	IM	If additional doses needed to prolong anesthesia provide 1/3 dose ketamine only	Minor surgery only; used for anesthetic induction when isoflurane used for anesthetic maintenance
Ketamine/	35 mg/kg	IM	If additional	Minor surgery only; used for anesthetic

Medetomidine/ Buprenorphine	0.5 mg/kg 0.03 mg/kg		doses needed to prolong anesthesia provide 1/3 dose ketamine only	induction when isoflurane used for anesthetic maintenance
<b>Reversal agents</b>				
Atipamezole	5X medetomidine (same <i>volume</i> as medetomidine)	IM preferred/ IV		More specific for reversal of medetomidine than xylazine
Yohimbine	0.2-1.0 mg/kg <sup>1</sup>	IV slowly/ IM	Once	Reverses sedative, cardiodepression, analgesic effects of xylazine
<b>Local anesthetics</b>				
Bupivacaine / Lidocaine mixture	1.5 mg/kg 0.5 mg/kg	SC	Once	Mix in same syringe for infiltration or local nerve block. Bupivacaine has slow onset (20-30 minutes), longer duration (4-8 hours); Lidocaine has rapid onset (5-10 minutes), shorter duration (1-2 hours).

## References

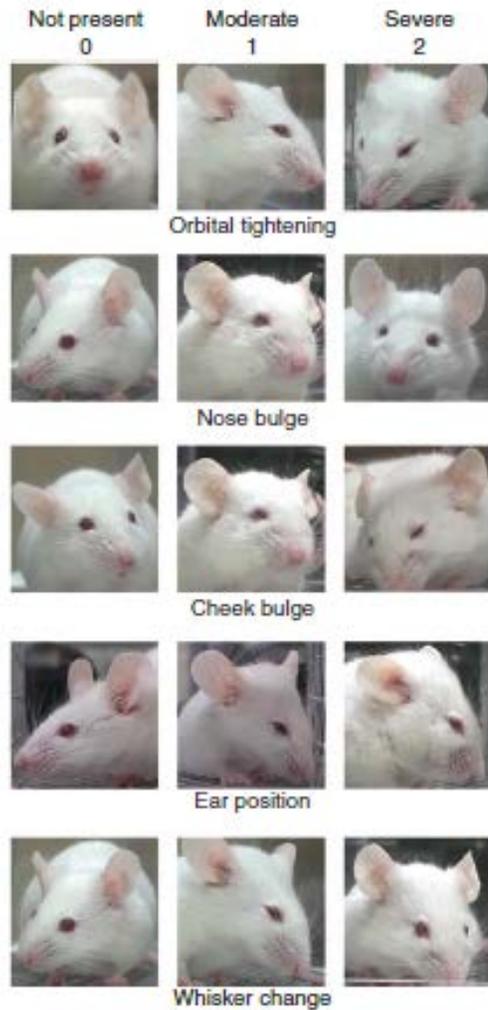
## Primary reference:

Fish RE et al. Anesthesia and Analgesia in Laboratory Animals, 2<sup>nd</sup> edition, 2008.

## Other references:

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2. (denoted by \* in table) Kuehn N. North American Companion Animal Formulary, 9th ed, 2010.
3. Swindle, MM. Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques, 2nd Ed, 2007.
4. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nature methods* 7:447-449.

Figure 1. Mouse Grimace Scale<sup>4</sup>



**Figure 1** | In the MGS, intensity of each feature is coded on a three-point scale. For each of the five features, images of mice exhibiting behavior corresponding to the three values are shown.

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