UNIVERSITY OF CALGARY	Animal Health Unit University of Calgary	**R1: Rat Anesthesia**
Version No.: 5.0		Effective Date: 12/6/2024
No. of pages: 13	Attachments: Y☒N ☐	Superseded Date: 8/7/2024
Level of Invasiveness: **B**		Prepared by: Animal Health Unit
Signed Copy Available Upon Request		

R1: Rat Anesthesia

***Note**: As this SOP has variations in methodology, ensure your specific methods are further explained within your Animal Use Protocol (AUP).*

PURPOSE: To outline the acceptable procedures for administering and monitoring anesthesia in adult rats.

RESPONSIBILITY: All students, staff and researchers trained by qualified staff under veterinary supervision

KEYWORDS: *anesthesia, anaesthesia, anesthetic, anaesthetic, rat, rats, isoflurane, ketamine, xylazine, drug, analgesia, analgesic*

Pre-Anesthetic Preparations

- These methods are to be used on adult rats that have been evaluated to be suitable for anesthesia.
- Ketamine is a controlled substance which requires a Section 56 Exemption from Health Canada to purchase and use in animal research. Application instructions can be found on the Animal Health Unit website at https://uofc.sharepoint.com/sites/spo-dept-vpr-ahu/SitePages/EXEMPTIONS.aspx .
- Isoflurane is a restricted substance which requires a veterinary prescription and a completed PI Responsibilities and Alternative Receivers form for purchase. Additional information can be found on the Animal Health Unit website at https://uofc.sharepoint.com/sites/spo-dept-vpr-ahu/SitePages/Prescriptions.aspx?csf=1&web=1&e=e2g9HY
- Always check the expiry date on anesthetic drugs and do not use expired products as the potency and sterility may be affected.
- Stock solutions must be diluted with sterile medical-grade, pyrogen-free water to obtain working drug solutions. See Appendix D for appropriate working concentrations of common injectable anesthetics. Drug mixtures may be stored at 4°C for up to 2 weeks.

Anesthesia Considerations and Monitoring

- Analgesia: **Rats undergoing anesthesia for the purpose of performing invasive or painful procedures (e.g. surgery) must be provided appropriate pain relief before, during, and after the procedure. Refer to SOP R2: Rat Analgesia.**

- Supportive Care During Anesthesia
  - Once the rat is anesthetized, ophthalmic ointment must be applied to both eyes to prevent corneal drying.
  - Heat must be provided throughout the anesthesia and recovery period, as rats lose body heat rapidly. A circulating water blanket set to 37ºC and covered with a cloth may be used.
    - Care must be taken to ensure the heat source does not burn the animal.
  - Supplementary oxygen can be beneficial throughout the procedure to prevent hypoxemia.
  - Once the animal is anesthetized, provide 10 mL/kg (4.5 mL for a 450g rat) of warmed (i.e. body temperature) fluids (medical-grade 0.9% Saline or Lactated Ringer's Solution) subcutaneously to help maintain body hydration and temperature.

- Anesthesia Monitoring
  - Depth of anesthesia must be monitored to ensure the animal is in an appropriate anesthetic plane. Table 1 outlines the planes of anesthesia in rats and the parameters for monitoring. See Appendix B for an Example Rodent Anesthesia Monitoring Sheet.
  - Vital signs (i.e. temperature, heart rate, respirations, mucous membrane colouration and reflexes) must be monitored throughout the anesthesia, at least every 5 minutes.
    - Pay close attention to changes in heart rate, body temperature and respiration.
  - If the plane of anesthesia is deemed inadequate for the procedure at any point, supplemental anesthetic is required. Surgical plane anesthesia is required for invasive procedures such as surgery (see Table 1).

Table 1: Rat Anesthetic Planes

Anesthetic Plane	Righting Reflex	Paw Withdrawal Reflex	Palpebral Reflex
Light	Absent	Present	Present
Surgical	Absent	Absent	Absent

- Post-Anesthesia Monitoring and Care:
  - Post-operative procedures must be followed for any animal recovering from anesthesia
  - Post-operative Monitoring:
    - Monitoring is to be recorded on the Rodent Anesthesia/Surgery Record cage card (see Appendix A) and in MOSAIC. These cage cards can be found in any of the ARC housing rooms. Detailed monitoring records can also be kept (see Appendix C).

        - Monitoring must continue throughout the recovery period. Do not leave the animal unattended until it is able to walk.
        - Ensure the animal's nose remains free from obstruction (e.g. the corner of the cage, bedding) as normal breathing can be inhibited.
        - Contact the University Veterinary staff if:
            - the animal appears painful or is excessively anxious or restless
            - the animal takes an unexpectedly long time (>30 minutes) to regain consciousness
            - any other abnormal behaviours are observed (see ROD10: Rodent Wellness and Pain Assessment)
    - Animals recovering from general anesthesia must be separated from cage mates until recovery is complete. Individual housing may be required beyond this time if animals begin to fight when recombined with their original cage mates.
        - Never combine animals from different cages.
    - Heat: External heat must be provided to all animals undergoing, and recovering from, general anesthesia. Ensure the heat source does not excessively heat the animal.
        - During recovery, the animal may be placed on a circulating water blanket set to 37ºC.
        - Rats should be recovered in a clean cage placed on a heat source (e.g. water blanket). Ensure that only half of the cage is on the heat source so the animal can move away from the heat if it becomes too warm.
    - Fluids: Animals recovering from anesthesia are often dehydrated. Providing 10mL/kg (4.5mL for a 450g rat) of warmed (i.e. body temperature), sterile, medical-grade subcutaneous fluids during anesthesia and/or recovery can help prevent organ damage.
        - Only use medical-grade fluids (0.9% saline or Lactated Ringer's Solution)

1. METHOD 1: Isoflurane Anesthesia

KEY POINTS:

- Isoflurane must be administered by an anesthesia machine with a calibrated isoflurane vaporizer. **'Drop jar anesthesia' (i.e. isoflurane soaked cotton balls placed in a closed container) is not permitted**, as this method is not able to provide reliable, consistent and safe levels of anesthesia.
  Anesthesia machines must be appropriately serviced, maintained, and used. For Health and Safety considerations, refer to the Helper Guide for Isoflurane Safety (Appendix E).
- Quick Dosing Reference:
  5% isoflurane for induction
  2% isoflurane for maintenance

MATERIALS

- Isoflurane
- Oxygen
- Calibrated anesthesia machine with appropriate scavenging system (e.g. F/AIR canister)
- Nose cone (small rodent size)

- Induction chamber
- Appropriate analgesic drugs
- Ophthalmic ointment
- Warmed (body temperature) medical-grade 0.9% Saline or Lactated Ringer's Solution
- Heat source

PROCEDURE:

1.1 Appropriate analgesia, supportive care, and anesthesia monitoring must be provided as outlined under Anesthesia Considering and Monitoring above.
1.2 Place the rat in the induction chamber and close the lid.
1.3 Set the oxygen flow meter to 1L/min or higher.
1.4 Set the vaporizer to 5% isoflurane.
1.5 Monitor the animal while anesthesia is induced. Once the animal is unable to right itself when the chamber is tipped, reduce the vaporizer to 2% isoflurane.
1.5.1. Turn the oxygen off before removing the animal from the induction chamber to minimize waste anesthetic gas exposure. Close the induction box immediately after the animal has been removed to minimize exposure to waste anesthetic gases. Promptly transfer the animal to a nose cone and turn the oxygen flow meter to 1L/min. The isoflurane should still be set to 2%.
1.6 Tape the circuit and nose cone to the table/bench to minimize their movement during the procedure.
1.7 When the procedure is finished, turn the vaporizer off (0%), but leave the oxygen flowing for up to 5 minutes to facilitate recovery. Move the rat into a sternal position (i.e. on its belly) to encourage quicker recovery Monitor the rat very closely during this period, as it may recover quickly.
1.8 Turn the oxygen flow meter off and remove the animal from the machine once its reflexes begin to return (e.g. positive toe pinch, presence of blink reflex, movement).
1.9 Place the animal in a clean recovery cage with heat support.
1.10 Turn off the main oxygen cylinder. Flush the anesthesia machine of residual gas.

2. METHOD 2: Injectable Anesthesia

KEY POINTS:

- The doses listed in the method will provide approximately 20-45 minutes of anesthesia. **These doses may not be sufficient to maintain animals within a <u>surgical</u> plane of anesthesia. Top-up administrations and/or other drugs (e.g., buprenorphine) may be required.**
- Survival procedures may benefit from using Ketamine doses at the lower end of the range, while the higher end is indicated for non-survival activities.
- Quick Dosing Reference:
  100mg/kg – 200mg/kg Ketamine
  10mg/kg Xylazine

MATERIALS:

- 1mL syringes
- 25g needles
- Drug stock solutions (ketamine, xylazine etc.)
- Sterile medical-grade, pyrogen-free water
- Sterile centrifuge tube or vials
- Appropriate analgesic drugs
- Ophthalmic ointment
- Warmed (body temperature) medical-grade 0.9% Saline or Lactated Ringer's Solution
- Heat source
- Oxygen and oxygen delivery system (optional)

PROCEDURE:

2.1 Appropriate analgesia, supportive care, and anesthesia monitoring must be provided as outlined under Anesthesia Conditions and Monitoring above.
  2.1.1 Where possible, provide the animal with supplemental oxygen throughout anesthesia.
2.2 Dilute the stock drugs/drug mixtures to appropriate working concentrations (see Appendix D).
2.3 Weigh the rat.
2.4 Using the working solution(s), prepare your drugs as per the dose specified in your ACC approved AUP.
  2.4.1 If not premixed, draw up drugs in separate, labelled syringes and safely recap the needles.
  2.4.2 Holding the syringe vertically, draw back on the plunger of the drug with the largest volume (typically ketamine) to create a large air bubble at the top of the syringe. Remove the larger volume needle.
  2.4.3 Uncap the needle of the drug with the smaller volume (typically xylazine) and carefully introduce the needle of the small volume solution into the syringe with the large volume solution.
  2.4.4 Dispense the small volume into the large volume syringe with one efficient movement of the plunger. This is done one at a time when combining more than 2 drugs.
  2.4.5 Reattach the larger volume needle to the mixed solution and prime the needle to remove air. Remember to re-label the syringe so it is apparent this is a mixed solution.
2.5 Administer the mixed solution by intraperitoneal injection. Please refer to R14: Rat Injections for recommended maximum volumes for IP injections.
2.6 If longer durations of anesthesia are required, administer a top-up dose of 30-50% the original dose when the animal begins to exhibit a withdrawal reflex. Do not continue the procedure until the supplemental anesthetic has taken effect and the appropriate anesthetic plane is achieved (Table 1). The top up dose will often take longer than the induction period to have effect.

Note: Please consult the University Veterinary Staff if you wish to consider reversal agents.

REFERENCES:

Canadian Council on Animal Care. CCAC Guidelines: Rats (2020). https://www.ccac.ca/Documents/Standards/Guidelines/CCAC-guidelines-on-husbandry-of-animals-in-science.pdf

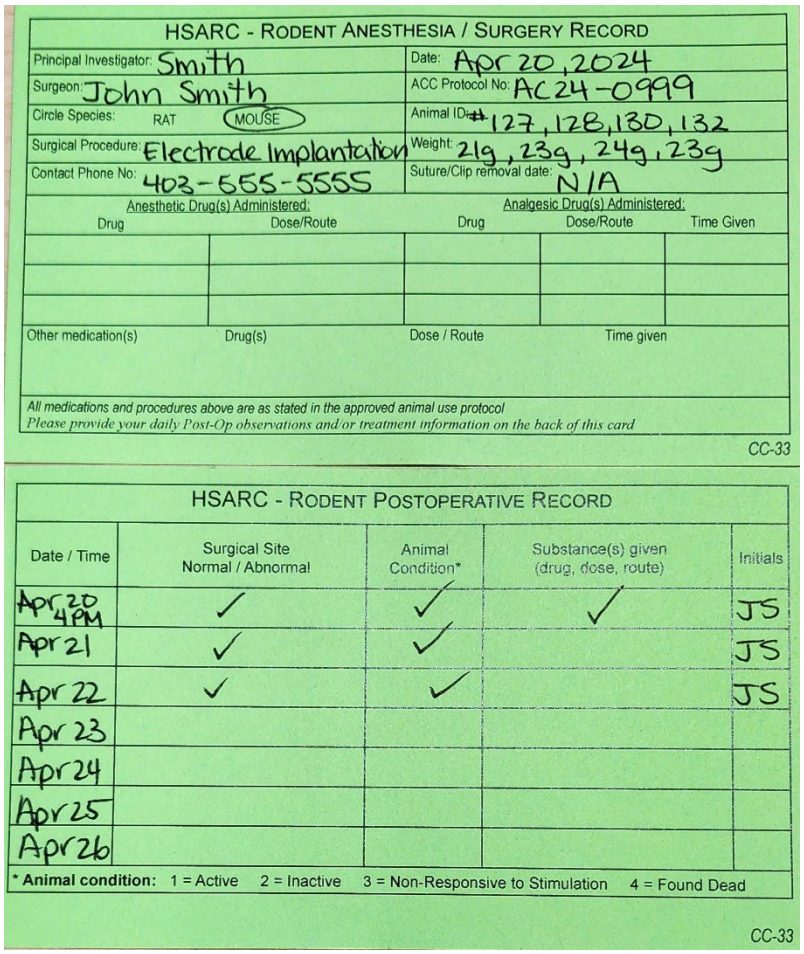
Carpenter, JW & Marion, C. 2018. *Exotic Animal Formulary*. 5th edition. Elsevier, Inc

Dyson et al. 2023. *Anesthesia and Analgesia in Laboratory Animals*, 3rd edition. Chapter 14 Anesthesia and analgesia in laboratory rodents. Elsevier, Inc

Flecknell, PA. 2023. *Laboratory Animal Anaesthesia and Analgesia*, 5th edition. Chapter 6 Anaesthesia of common laboratory species. Academic Press, Inc

APPENDIX A: Example Rodent Anesthesia and Surgery Cage Card (HSARC)

This card should be used to indicate any type of anesthesia or survival surgery procedure that has occurred as well as the subsequent monitoring associated with the procedure.



HSARC - RODENT ANESTHESIA / SURGERY RECORD

Principal Investigator: Smith	Date: Apr 20, 2024
Surgeon: John Smith	ACC Protocol No: AC24-0999
Circle Species: RAT (MOUSE)	Animal ID#: 127, 128, 130, 132
Surgical Procedure: Electrode Implantation	Weight: 21g, 23g, 24g, 23g
Contact Phone No: 403-555-5555	Suture/Clip removal date: N/A

Anesthetic Drug(s) Administered: Drug	Dose/Route	Analgesic Drug(s) Administered: Drug	Dose/Route	Time Given

Other medication(s)	Drug(s)	Dose / Route	Time given

All medications and procedures above are as stated in the approved animal use protocol
Please provide your daily Post-Op observations and/or treatment information on the back of this card

CC-33

HSARC - RODENT POSTOPERATIVE RECORD

Date / Time	Surgical Site Normal / Abnormal	Animal Condition*	Substance(s) given (drug, dose, route)	Initials
Apr 20 4PM	✓	✓	✓	JS
Apr 21	✓	✓		JS
Apr 22	✓	✓		JS
Apr 23				
Apr 24				
Apr 25				
Apr 26				

*** Animal condition:** 1 = Active 2 = Inactive 3 = Non-Responsive to Stimulation 4 = Found Dead

CC-33

How to Use:

1. Fill in <u>ALL</u> the information presented in the top front portion of the card, including Principal Investigator, Date, Protocol #, Surgeon, Species, Emergency Phone #, Animal ID(s), Baseline Body Weights, Surgical Procedure, and Suture/Clip Removal Date (if applicable).
2. On the back of the card, prefill the dates/times of the scheduled monitoring as described in the approved Animal Use Protocol.
3. Initial and check off the parameters that were observed <u>ONLY</u> when the monitoring is complete.
4. The anesthesia and surgical exams which contain the anesthesia, analgesia and procedure details are added into Mosaic on the date of the procedure.
5. Post-procedural treatments administered and monitoring (e.g. surgical site, animal condition/clinical score, body weights) are entered into Mosaic at the time of the monitoring.

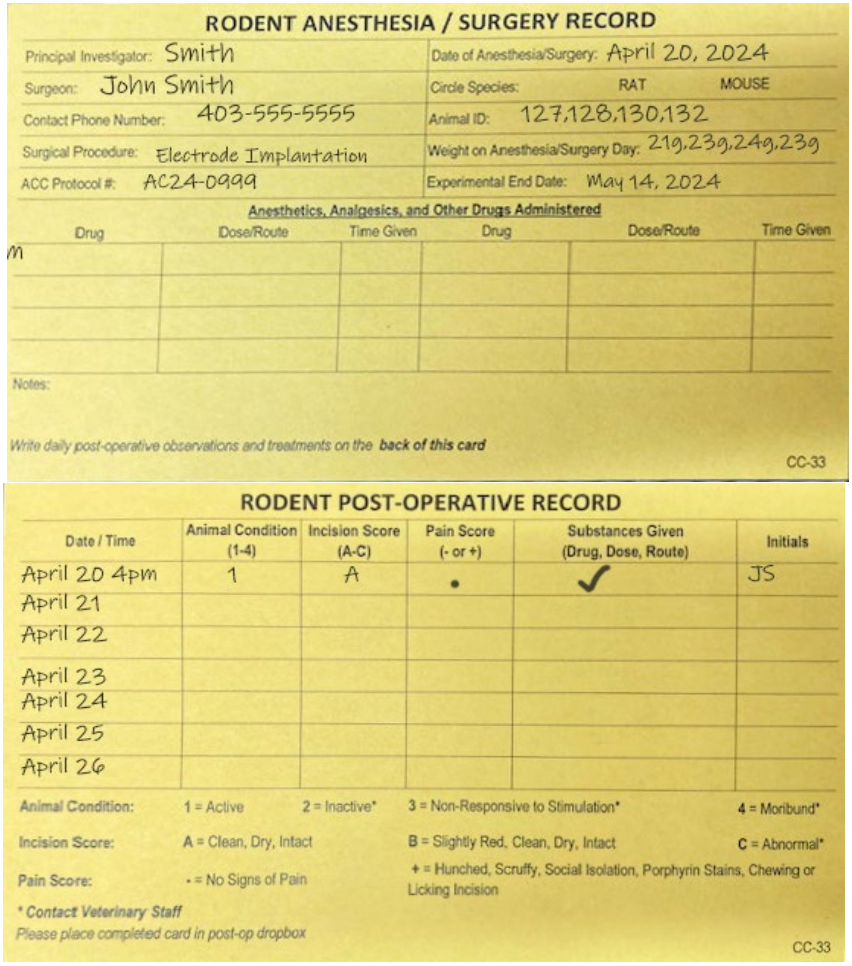
Notes:
If you require ARC Staff to administer analgesic drugs or provide monitoring on your behalf, it must be described in the Animal Use Protocol and be arranged with the ARC Facility Manager ***before*** the scheduled procedure.

If the surgeon and the emergency contact are different people, the name of the contact responsible for post-operative monitoring must also be included.

If animal condition is assessed at a 2 or 3, ARC Staff and Veterinarians must be notified.

APPENDIX A (Continued): Example Rodent Anesthesia and Surgery Cage Card (LESARC)

*Refer to previous page for instructions for how to use



RODENT ANESTHESIA / SURGERY RECORD

Principal Investigator: Smith	Date of Anesthesia/Surgery: April 20, 2024
Surgeon: John Smith	Circle Species: RAT MOUSE
Contact Phone Number: 403-555-5555	Animal ID: 127,128,130,132
Surgical Procedure: Electrode Implantation	Weight on Anesthesia/Surgery Day: 219,239,249,239
ACC Protocol #: AC24-0999	Experimental End Date: May 14, 2024

Anesthetics, Analgesics, and Other Drugs Administered

Drug	Dose/Route	Time Given	Drug	Dose/Route	Time Given
m					

Notes:

Write daily post-operative observations and treatments on the ***back of this card***

CC-33

RODENT POST-OPERATIVE RECORD

Date / Time	Animal Condition (1-4)	Incision Score (A-C)	Pain Score (- or +)	Substances Given (Drug, Dose, Route)	Initials
April 20 4pm	1	A	-	✓	JS
April 21					
April 22					
April 23					
April 24					
April 25					
April 26					

Animal Condition: 1 = Active 2 = Inactive* 3 = Non-Responsive to Stimulation* 4 = Moribund*

Incision Score: A = Clean, Dry, Intact B = Slightly Red, Clean, Dry, Intact C = Abnormal*

Pain Score: - = No Signs of Pain + = Hunched, Scruffy, Social Isolation, Porphyrin Stains, Chewing or Licking Incision

** Contact Veterinary Staff*

Please place completed card in post-op dropbox

CC-33

APPENDIX B: Example Rodent Anesthesia Monitoring Sheet

Name: _____ Date: _____

Animal ID: _____ Animal Weight (g):_____

Procedure: _____

□ Injectable anesthesia:	□ Inhalational anesthesia:
• Ketamine (full dose): _____ • Xylazine (full dose): _____ • Ketamine (top-up): _____ Xylazine (top-up): _____	• Induction: 5% isoflurane • Maintenance: 2% isoflurane

□ Other substances administered (dose, volume, route, time):

- Analgesics: _____
- Fluids: _____
- Other: _____

Time of Induction: _____ Time of Recovery: _____

Time	HR (beats/min) Or pattern	RR (breaths/min) Or pattern	Temp (°C)	Blink reflex (+/-)	Pedal reflex (+/-)	Tissue colour	Comments

Rat Anesthesia Vital Parameters *Check every 5 minutes*	
Respiratory rate (RR)*	70-110 breaths/min (drop of 50% normal during anesthesia)
Respiratory pattern	Deep, shallow, slow, rapid
Heart rate (HR)*	250-500 beats/min
Pulse pattern	Unable to count, able to count, faint, strong
Temperature*	35.9 – 37.5°C
Tissue colour (nose, ears, paws)	Pink, Pale, Blue, Yellow, Red

** Determined using Rodent PhysioSuite*

APPENDIX C: Rodent Postoperative Monitoring Sheet

Date & Time	**Weight** (g)	**Food**	**Water**	**Urine / Fecal Output**	**Incision (If applicable)**	**Pain Assessment**	**Analgesic(s) or Drug(s) Given** (complete drug name, dose, route)	**Comments**	**Initial**
Immediately Post-op	*Pre-Anesthesia or Surgery*	*Offered:*	*Offered:*	*Urine:* *Feces:*	*Location?* *Method of closure?*		*Pre-meds?* *Additional meds?*	*Time of Recovery?*	
		Consumed: *Offered:*	*Consumed:* *Offered:*	*Urine:* *Feces:*	*Appearance?*				
		Consumed: *Offered:*	*Consumed:* *Offered:*	*Urine:* *Feces:*	*Appearance?*				

APPENDIX C (Continued): Example Rodent Postoperative Monitoring Sheet Filled Out

Date & Time	**Weight** (g)	**Food**	**Water**	**Urine / Fecal Output**	**Incision (if applicable)**	**Pain Assessment**	**Analgesic(s) or Drug(s) Given** (complete drug name, dose, route)	**Comments**	**Initial**
Immediately Post-op May 12/24 10:30am	*Pre-anesthesia or Surgery* 310	*Offered:* - 10 pellets - ¼ can recovery gel - 1 tsp sunflower seeds	*Offered:* 500 ml	*Urine:* 0 *Feces:* -	*Location?* Right neck *Method of closure?* Absorbable PDS sutures	Quiet, moves after gentle nudge, not responsive to gentle palpation of surgical site; Not painful	*Pre-meds?* Meloxicam 2mg/kg SC Buprenorphine 0.05mg/kg SC *Additional meds?* Sterile saline 2ml SC + 1ml intra-incisional	*Time of Recovery?* 10:15 am Under anesthesia for 2h Took 20min to recover	AZ/DJ
May 12/24 3:30pm	Not examined	*Consumed:* - 0 pellets - 1/8 can recovery gel - 0 sunflower seeds *Offered:* - 10 pellets - - 1/8 can recovery gel - 1 tsp sunflower seeds	*Consumed:* 10 ml *Offered:* 490 ml	*Urine:* 1 *Feces:* +	*Appearance?* Intact, some dried blood	Quiet, alert, responsive to gentle nudge, ambulating occasionally, hunched posture, scruffy coat, some reaction to palpation of surgical site; Painful	Buprenorphine 0.05mg/kg SC	Some clear discharge with blood. Cleaned incision	AZ
May 13/24 8:30am	308	*Consumed:* - 2 pellets - 1/8 can recovery gel - All seeds *Offered:* -10 pellets	*Consumed:* 20ml *Offered:* 470ml	*Urine:* 2 *Feces:* ++	*Appearance?* Dried blood, 0.5cm opening, mild swelling	Bright, alert, moving around cage, porphyrin on paws, no reaction to palpation of incision; No painful	Meloxicam 2mg/kg SC Buprenorphine 0.05mg/kg SC	Cleaned incision and closed opening with tissue glue 3% decrease in body weight. Continue monitoring.	AZ

APPENDIX D: Drug Dilutions for Rat Anesthesia – How to Achieve a Working Solution

Drug (STOCK Concentration)	Dilution Factor	Dilution (Parts Drug : Parts Sterile Water)	Final Concentration of WORKING Solution
Ketamine (100mg/mL)	4	1:3	25 mg/mL
Xylazine (100mg/mL)	20	1:19	5 mg/mL
Xylazine (20mg/mL)	4	1:3	5 mg/m?
Ketamine and Xylazine (100:10 premix)	10	1:9	Ketamine 10mg/mL; Xylazine 1mg/mL
Ketamine and Xylazine (200:10 premix)*	10	1:9	Ketamine 20mg/mL; Xylazine 1mg/mL

* Used only for non-survival procedures

Example Dilution:

Goal: Dilute 100mg/mL ketamine to a working concentration of 25 mg/mL ketamine (4x dilution).

Formula: $C1V1 = C2V2$

Adjusted formula: $V1 = \frac{C2V2}{C1}$

$C1$ = 100mg/mL ketamine
$V1$ = X mL of ketamine (100mg/mL)
$C2$ = 25mg/mL ketamine
$V2$ = 5mL of ketamine (25mg/mL)

$$V1 = \frac{25\left(\frac{mg}{mL}\right) \text{ x } 5(mL)}{100\frac{mg}{mL}} \Rightarrow V1 = \frac{125\text{ mg}}{100\frac{mg}{mL}} \Rightarrow V1 = 1.25\text{mL of ketamine (100mg/mL)}$$

To achieve a final volume of 5mL:

- Add 1 part ketamine to 3 parts sterile water.
    - To achieve a final volume of 5mL, this equates to 1.25mL ketamine (100mg/mL) added to 3.75mL sterile water.
    - Final concentration of working ketamine solution = 25mg/mL

APPENDIX E: Helper Guide for Isoflurane Safety

UNIVERSITY OF CALGARY

October 2024

Helper Guide for Isoflurane Safety

To enhance safety for personnel working with isoflurane this document has been developed by the Animal Health Unit in conjunction with Environment, Health and Safety. Principal Investigators (PIs) are ultimately responsible for informing workers about the hazards associated with isoflurane handling and ensuring workers receive sufficient training to conduct work safely.

General Considerations

- PIs/labs to include working with isoflurane in Hazard Identification, Assessment, and Control Form (HACF)
- For workers handling isoflurane training resources include:
  - The IAUTP- Rodent Anesthesia course (online and in-person training components)
  - On-site training by trained and competent lab personnel
- Workers must be supervised until deemed trained and competent to perform work safely without supervision.
- PIs/labs capture working with isoflurane in their own task-specific standard operating procedure (SOP).
- Hazard Assessment and Recommended Controls
  - During the onboarding process inform personnel on how to work safely with isoflurane.
  - EHS is available for site visits on request to review procedures, controls, etc.
- The use of fume hoods is intended for particulate and vapour use.
- Biosafety Cabinets are intended for particulate use.

Isoflurane Health Hazards

- Eye irritation
- Specific target organ toxicity through inhalation (central nervous system, cardiovascular system)
- Acute effects
  - Low exposure: may cause nausea, headache, drowsiness, or dizziness.
  - Overexposure/high exposure: may cause anesthesia, respiratory depression, and coughing.
- Teratogenic effects in pregnant people or people of childbearing age, can cause miscarriage and malformations.

Spill Response

- Follow UCalgary Spill Response Procedure
- For large spills, evacuate and secure the area as high concentrations of isoflurane can lead to unconsciousness and death. Contact Campus Security to report.

First-aid measures

- Call 911 if in critical, life-threatening conditions.
- Contact Campus Security (403-220-5333) if medical attention is required (non-life-threatening conditions)