UBC ANIMAL CARE COMMITTEE
TECH 17 - Analgesia for Adult Mice and Rats
Buprenorphine SOP

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PURPOSE:

- To describe the procedure for administering an opioid drug in adult mice and rats to provide pain relief (analgesia) in cases of moderate to severe pain.
- This Standard Operating Procedure (SOP) describes the use of one type of opioid, buprenorphine (brand names: Temgesic® or Buprenex®).
- This SOP follows the UBC Surgical Class and Analgesia Guidelines and is in keeping with the Canadian Council on Animal Care (CCAC) current guidelines on the use of analgesics.

RESPONSIBILITY:

- Those trained UBC Persons listed on an approved Animal Care Committee (ACC) Animal Care Protocol who are responsible for performing the procedures requiring the administration of buprenorphine.
- All animal users performing analgesic injections in rodents must have successfully completed the UBC Animal Care Services (or equivalent) Introduction to Working with Rodents in Research (IWRR), and Rodent Restraint/SQ/IP injections (RSCIP) courses.

MATERIALS: (**can be purchased from Animal Care Services*)

- Buprenorphine injectable (0.3 mg/ml) (i.e.: Temgesic®, Buprenex®)
- *Sterile syringes (0.3 -1 ml)
- *Sterile needles (25-27 G, 1/2" or smaller)
- *Sterile GLASS, amber, multi-use vial for diluted solutions
- *Sterile 0.9% normal saline (Sodium chloride, NaCl; for dilution)
- *Sharps container
- Weigh scale
- *Sterile Lactated Ringer's solution or 0.9% sterile saline (if giving subcutaneous (SQ) fluids for dehydration)
- *Sterile 1-10 ml syringes (if giving SQ fluids for dehydration)

Table 1 - DOSE FOR MICE AND RATS:

Species	Dose*	Concentration to Dilute to*	Frequency*
Rat	0.01 – 0.05 mg/kg	0.03 mg/ml	Every 6-12 hours
Mouse	0.05 – 0.1 mg/kg	0.015 mg/ml	Every 6-12 hours

*See next page for dilution instructions. See Table 3 for suggested starting doses and frequencies based on type of procedure performed.

PROCEDURE:

1. Weigh animal(s) to be treated.
2. Calculate the dose and volume in ml of buprenorphine required (refer to Table 1).
    a. Refer to Table 3 for suggested starting dose and frequency of administration based on the procedure being performed and degree of pain expected.
    b. See instructions on how to calculate below.
    c. Injectable formulation may require dilution. See dilution instructions below.
3. Draw up the calculated dose in a sterile syringe.
    a. Use a new sterile syringe and needle for each animal.
4. Gently restrain the mouse or rat in the best appropriate manner for a subcutaneous injection.
5. Administer the dose of buprenorphine subcutaneously in the loose skin at the base of the neck or over the rump (hips) of the animal.
6. If animal(s) are, or may become dehydrated, administer sterile 0.9% sodium chloride or Lactated Ringers solution subcutaneously (SQ).
    a. See UBC ACC Guidelines and SOP for the Maintenance of Fluid Homeostasis in Animals for details. A useful starting point is 20 ml/kg SQ.
7. Reassess for signs of pain at least every 6-8 hours.
    a. If still painful, then alternative analgesics may be required (e.g. Meloxicam).
    b. If no improvement is observed after administration of analgesia, contact the Principal Investigators and/or the facility's Clinical Veterinarian.

DILUTION INFORMATION:

Buprenorphine (0.3 mg/ml) requires dilution if the calculated dose is less than 0.05 ml to accurately dose mice and small rats (< 160 g).

- **Rats:** Prepare a 1:10 dilution of 0.3 mg/ml buprenorphine with sterile 0.9% saline
    - Final concentration will be 0.03 mg/ml.
    - Add 1 part buprenorphine (0.3 mg/ml) to 9.0 parts sterile 0.9% saline for a total volume of 10 parts of diluted solution.
        - E.g. Add 1.0 ml Buprenorphine (0.3 mg/ml) to 9.0 ml sterile 0.9% saline for a total volume of 10 ml of diluted solution.
- **Mice:** Prepare a 1:20 dilution of 0.3 mg/ml buprenorphine with sterile 0.9% saline
    - Final concentration will be 0.015 mg/ml.
    - Add 1 part buprenorphine (0.3 mg/ml) to 19 parts sterile 0.9% saline for a total volume of 20 parts of diluted solution.
        - E.g. Add 0.5 ml of 0.3 mg/ml buprenorphine to 9.5 ml sterile 0.9% saline for a total volume of 10 ml of diluted solution.
- Label vial with drug name, concentration and date of dilution. It is suggested to also add the initials of the person who made the dilution.
- Store diluted solution aseptically in a sterile, GLASS, amber, multi-dose vial and protect from light.
- Diluted solutions must be discarded within 30 days from date of dilution.

- Follow the instructions provided by Health Canada on the exemption approval paperwork for proper disposal.

CALCULATING DRUG VOLUME (IN ML) TO BE ADMINISTERED:

- Convert animal's weight from grams to kilograms
  - Divide the weight in grams by 1000
    - E.g. 25g mouse ÷ 1000 = 0.025kg
- Calculate the volume to give in ml
  - Volume (ml) = [dose(mg/kg) x animal's weight(kg)] ÷ drug concentration(mg/ml)
    - E.g. For a 25g mouse getting a dose of 0.05 mg/kg of 0.015 mg/ml buprenorphine
    - Volume = (0.05mg/kg buprenorphine x 0.025kg) ÷ 0.015mg/ml = **0.08 ml**

Table 2 - Examples of Dosing

Weight of **Rat**	Buprenorphine **diluted to <u>0.03 mg/ml</u>** Dose: 0.01 – 0.05 mg/kg
250 g (0.25 kg)	0.08 – 0.42 ml
350 g (0.35 kg)	0.12 – 0.6 ml
450 g (0.45 kg)	0.15 – 0.75 ml
550 g (0.55 kg)	0.18 – 0.9 ml
Weight of **Mouse**	Buprenorphine **diluted to <u>0.015 mg/ml</u>** Dose: 0.05 – 0.1 mg/kg
25 g (0.025 kg)	0.08 – 0.17 ml
35 g (0.035 kg)	0.12 – 0.24 ml
45 g (0.045 kg)	0.15 – 0.3 ml
55 g (0.055 kg)	0.18 – 0.36 ml

CHOOSING A DOSE AND FREQUENCY OF ADMINISTRATION OF BUPRENORPHINE:

- Dose and frequency of buprenorphine administered should be based on the degree of pain expected, the degree of pain observed and any side effects that are noted.
- See Table 3 for examples of doses and frequencies of administration recommended for common research procedures.
  - Contact your clinical veterinarian for tailored dose and frequency.
- If moderate pain is expected, start with a low to mid-dose range; if severe pain is expected, start with the higher end of the dose range
  - Reassess based on clinical signs of pain and behavior.
- With the higher doses for invasive surgery, it is acceptable to give ½ the calculated dose at the time of anesthesia induction and the other ½ when the animal begins to recover from the anesthesia (is beginning to move around the cage).
  - Calculate the time for the next dose from the time the second ½ dose was given.
- For the moderate and severe pain categories, it is recommended to slowly wean down the dose of buprenorphine given daily beginning the day after surgery.

- Give the animal the full dose the morning of the day after surgery and if the animal is doing well and the pain is controlled, reduce each following dose by approximately 10% until the dose is too small to measure accurately.

Table 3 - Choosing a dose and frequency of administration of Buprenorphine

Pain Classification Examples				
Class	1	2	3	4
Pain Level	Mild Pain	Moderate Pain	Moderate/Severe Pain	Severe Pain
Examples	Subcutaneous implant with trocar	Craniotomy +/- Implant	Laparotomy with major organ manipulation or removal	Peritonitis/pancreatitis
	Ocular procedures	Simple laparotomy	Organ Transplant	Limb ischemia
	Intratracheal injections	Embryo transfer	Spinal surgery	Spinal cord or nerve damage
	Skin biopsy/wound	Ovariectomy	Thoracotomy (costal approach)	Thoracotomy (sternal approach)
	Peripheral vessel cut down or cannulation	Castration	MCAO	Cecal ligation and puncture
	Intramuscular injection	Intra-peritoneal osmotic pump		Orthopedics/Fractures
	Subcutaneous osmotic pump			Bone cancer
	Dental extraction			
Recommended Rat Dose	0.01 mg/kg	0.02 mg/kg	0.04 mg/kg	0.05 mg/kg
Recommended Mouse dose	0.05 mg/kg	0.06 mg/k	0.08 mg/kg	0.1 mg/kg
Frequency of administration	Twice a day (every 12 hours) the day of surgery	Twice to three times a day (every 8-12 hours) for two days and depending on pain assessment	Twice to three times a day (every 8-12 hours) for 3 days and depending on pain assessment	Three times a day (every 6-8 hours) for at least 3 days and depending on pain assessment

IMPORTANT NOTES:

- Buprenorphine gives 6-12 hours of analgesia in rodents so it is the recommended opioid for treatment of moderate to severe pain in rodents. Other opioid analgesics, such as morphine and hydromorphone, have a much shorter duration of action so would need to be dosed much more frequently (up to every 2 hours). The use of shorter acting opioids should be discussed with a Clinical Veterinarian prior to use.
    - Sustained release Buprenorphine is also available and will provide analgesia for approximately 24-48 (up to 72) hours with one injection. Unfortunately, it is not available as an exempted drug from Health Canada. Contact your Clinical Veterinarian for information on using the sustained release formulation.
- Buprenorphine is a controlled drug and an exemption must be obtained from Health Canada to be allowed to order and use this drug.
    - Information on the application for exemption for scientific purposes can be found here: https://www.canada.ca/en/health-canada/services/health-concerns/controlled-substances-precursor-chemicals/exemptions/application-form-exemption-use-controlled-substance-scientific-purposes.html
    - A controlled drug log must be kept following the requirements stipulated by Health Canada as part of the exemption.

    - The controlled drug must be stored, recorded and discarded following the requirements of Health Canada.

- Do not use phosphate buffered saline (PBS) or sterile water for dilution; PBS is not equivalent to normal saline.
- Store diluted buprenorphine in glass, not plastic vials, and away from light.
    - Drug will degrade in the prolonged presence of light so store either in amber vials, foil-wrapped vials, or stored in a dark cabinet.
    - Drug binds or reacts to plastic, thereby reducing its efficacy.
- When possible, pain must be treated pre-emptively (before the cause).
- An adequate analgesic plan must be described in the approved Animal Care Protocol for prevention and treatment of pain associated with the experimental procedures.
- For spontaneous or unexpected pain, Principal Investigators and the Clinical Veterinarian should be consulted immediately and prior to administration of analgesics so that an appropriate pain management plan can be devised.

COMPLICATIONS:

- Pica (consumption of non-food items such as bedding or nesting)
    - ***Cause:*** More frequently seen in rats receiving higher doses of buprenorphine to control severe pain. The consumption of bedding or nesting can be severe enough to cause obstruction of the esophagus.
    - ***Clinical signs:*** Trouble breathing (dyspnea), open mouth breathing or salivation.
    - ***Response*****:** Remove the bedding and nesting, place in a warm cage with oxygen administration and contact a Clinical Veterinarian immediately.
- Changes to Gastrointestinal motility
    - ***Cause:*** Opioids can cause gut stasis (decreased gut movement)
    - ***Clinical signs***: Decreased appetite, weight loss, dehydration (sunken eyes, loss of skin elasticity), or constipation.
    - ***Response:*** Provide 20 ml/kg subcutaneous fluids (Lactated Ringer's Solution or 0.9% Sodium Chloride) and safe supplemental heat. Contact a Clinical Veterinarian to discuss possibly changing the dose or frequency of administration.
- Hyperactivity or sedation
    - ***Cause:*** Though not commonly seen in rodents, overdosing with opioids can result in hyperactivity.
    - ***Clinical signs:*** Increased activity during the day, the tail held straight and abnormally high above the bedding and decreased nest building.
    - ***Response:*** Provide 20 ml/kg subcutaneous fluids (Lactated Ringer's Solution or 0.9% Sodium Chloride) and safe supplemental heat. Contact a Clinical Veterinarian to discuss possibly changing the dose or frequency of administration (e.g. reducing the next dose by 10%)

REFERENCES: (https://animalcare.ubc.ca/animal-care-committee/sops-policies-and-guidelines)

- UBC SOP Subcutaneous Injections in Rats and Mice
- UBC Rodent Anesthesia and Analgesia Formulary and General Drug Information
- UBC Surgical Class and Analgesia Guidelines
- UBC ACC Guidelines and SOP for the Maintenance of Fluid Homeostasis in Animals