UBC ANIMAL CARE COMMITTEE
TECH 16 – Local Anesthesia/Analgesia in Adult Mice and Rats
Bupivacaine SOP

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PURPOSE:

- To describe the procedure for administering a local anesthetic in adult mice and rats to provide pain relief locally (local anesthesia and analgesia) in cases of mild to severe pain (such as the site of a skin incision).
- This Standard Operating Procedure (SOP) describes the use of one type of local anesthetic drug, bupivacaine (brand name: Marcaine®).
- 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 procedures requiring the administration of local anesthesia/analgesia.
- All animal users performing local anesthetic injections in rodents must have successfully completed the UBC Animal Care Services (or equivalent) Introduction to Working with Rodents in Research (IWRR), Rodent Restraint/SQ/IP injections (RSCIP), Rodent Anesthesia (RA), and Rodent Surgery (RSx) courses.

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

- Bupivacaine injectable 0.5% (5mg/ml) (i.e. Marcaine®)
- Sterile syringes (0.3-1 ml)
- Sterile needles (25-27 G, 1/2" or smaller)
- Sterile, amber, multi-use vials for diluted solutions (protect from light)
- Sterile 0.9% normal saline (Sodium chloride, NaCl; for dilution)
- Sharps container
- Weigh scale

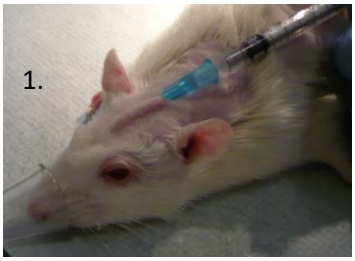
Table 1 - DOSE FOR MICE AND RATS:

Local Anesthetic	Maximum Dose	Diluted Concentration	Comment
Bupivacaine (Marcaine) (0.5%; 5 mg/ml)	Do not exceed 8 mg/kg total dose	Dilute to 0.25% (2.5 mg/ml)	Onset of action: 10 minutes Duration of action: 4-8 hours

See below for dilution instructions

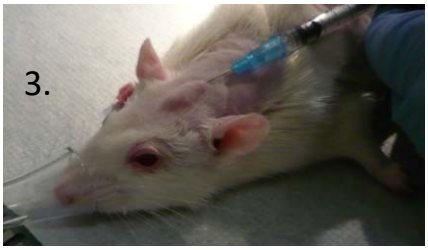
PROCEDURE:

1. Weigh animal(s) to be dosed.
2. Dilute the Bupivacaine in order to accurately dose mice and rats.
    a. See instructions on how to calculate below.
3. Calculate the maximum dose and volume in ml of diluted Bupivacaine required (refer to table 1).
    a. See instructions on how to calculate dose below.
    b. This is the maximum volume that can be safely used per animal.
    c. If small incisions are planned, use only the volume needed to deposit the local anesthetic along the planned incision line(s).
    d. If multiple incisions per animal are planned, the maximum calculated dose should be divided between all the incisions.
4. Draw up the required volume in a sterile syringe. Do not exceed the maximum calculated dose. See Examples in Table 2.
    a. Use a new sterile syringe and needle for each animal.
5. Shave and clean the animal's skin in preparation for surgery (see UBC Rodent Survival Surgery SOP for details).
6. Administer the calculated dose of Bupivacaine as a line block subcutaneously (SQ) along the planned incision site(s). See Figures 1 to 3 below.



Insert needle fully into the SQ space below the planned incision site. Gently pull back syringe plunger to ensure no blood in hub of needle (remove needle and reattempt if blood seen).

Begin injecting small volumes of the local anesthetic as the needle is withdrawn.
A "bleb" should form.



Complete injection of the local anesthetic under the planned incision site.
If incision site is longer than needle length, re-insert needle further along the planned incision and repeat with remaining volume.

DILUTION INFORMATION:

Bupivacaine (0.5%) requires dilution to accurately dose mice and rats.

- Prepare a 1:2 dilution of 0.5% (5 mg/ml) Bupivacaine with sterile 0.9% saline.
    - Final concentration will be 0.25% (2.5 mg/ml) or half the strength.
    - Add the equivalent volume of saline as volume of bupivacaine (1 part saline to 1 part bupivacaine).

        - E.g. Add 1.0 ml Bupivacaine (5 mg/ml) to 1.0 ml sterile 0.9% saline for a total volume of 2 ml of diluted solution.
- Store diluted solution aseptically in a sterile, amber, multi-dose vial. Protect from light. If amber vials are not available, wrap in tin foil.
- 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.
- Diluted solutions must be discarded within 30 days from date of dilution.

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
    - = [dose (mg/kg) x weight of animal (kg)] ÷ concentration of drug (mg/ml)
    - E.g. For a 25g mouse receiving a dose of 7 mg/kg of 2.5 mg/ml Bupivacaine
      Volume (ml) = (7 mg/kg Bupivicaine x 0.025kg) ÷ 2.5 mg/ml = **0.07 ml**

Table 2 - Examples of Dosing

Weight of **Rat**	Bupivacaine **diluted to 2.5 mg/ml** **Maximum dose 8 mg/kg**
250 g (0.25 kg)	0.8 ml
350 g (0.35 kg)	1.12 ml
450 g (0.45 kg)	1.44 ml
550 g (0.55 kg)	1.76 ml

Weight of **Mouse**	Bupivacaine **diluted to 2.5 mg/ml** **Maximum dose 8 mg/kg**
25 g (0.025 kg)	0.08 ml
35 g (0.035 kg)	0.11 ml
45 g (0.045 kg)	0.14 ml
55 g (0.055 kg)	0.17 ml

IMPORTANT NOTES:

- Local anesthetics work by blocking pain signals reaching the central nervous system (brain and spinal cord).
    - In rodents, they are commonly given at surgery to provide pain relief to the skin.
        - Local anesthetics are given before the first incision and are typically given in conjunction with systemic analgesics.
        - Dripping the local anesthetic into the incision after the incision is already made is less effective than the line block described above, but is still better than not using a local anesthetic.
    - They have few systemic effects but special care must be taken in small animals, such as mice and rats, as it can be easy to exceed the toxic dose.

    - If working with neonates, consult with your Clinical Veterinarian.
- 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.
- Do not use phosphate buffered saline (PBS) for dilution; PBS is not equivalent to normal saline.
- Intramuscular and intravenous injection must be avoided in rodents.
- Side effects of local anesthetics are few but can include systemic toxicity due to overdosing or accidental intravenous injection (see Complications below).
- The maximum volume is the maximum volume per animal, not per site of injection.
    - Use the smallest volume required to administer the line block beneath the planned incision and do not exceed the maximum volume calculated.

COMPLICATIONS:

- Systemic toxicity
    - ***Cause:*** These signs can be seen if more than the maximum dose is administered or by accidental dosing by intravenous injection.
    - ***Clinical signs:*** Seizures, heart rhythm disturbances, drop in blood pressure and death
    - ***Response:*** Discontinue drug, Provide 20 ml/kg subcutaneous fluids (Lactated Ringer's Solution or 0.9% saline) and safe supplemental heat. Provide supplemental oxygen if the animal is showing open mouth breathing. Contact a Clinical Veterinarian.

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 Rodent Survival Surgery Policy and SOP
- UBC ACC Guidelines and SOP for the Maintenance of Fluid Homeostasis in Animals