UNIVERSITY of GUELPH

Animal Care Services

Title:
Administration of pain control to rats and mice

SOP.ACS.814.Administration of pain control to rats and mice

Approval Date: April 14, 2023 **Revision Date: June 9 2023**

1. ***Purpose:*** To provide instructions for the administration of different formulations of analgesics to mice and rats for pain control and to meet or exceed the standards as set out in the CCAC Guide to the Care and Use of Experimental Animals.

2. ***Responsibilities:*** Animal care staff, veterinarians, and trained individuals listed on an approved Animal Utilization Protocols (AUPs). All animal users administering drugs to mice and rats must have successfully completed Mouse/Rat A/B training courses.

3. ***Introduction:*** Rats and mice should be provided with analgesia for painful procedures and as directed by the Animal Care Committee (ACC), both to prevent suffering and to decrease scientific variability associated with distress. The most used analgesics in laboratory rodent medicine are non-steroidal anti-inflammatories (NSAIDS), opioids, and local anesthetics.

   Planned use of analgesics for experimental purposes (e.g., for surgery) must be outlined in the Animal Utilization Protocol (AUP) procedure section. Analgesics may also be recommended by a veterinarian as part of a treatment plan.

4. Procedures:

GUIDING PRINCIPLES OF PAIN CONTROL

- For mild pain, a single analgesic agent may be used, i.e., either an opioid or an NSAID
    - E.g., subcutaneous implant, intramuscular injection, cancer cell injection, tracheal injections, skin biopsy
- For moderate-severe pain, a multi-modal analgesic protocol that includes both an NSAID and an opioid should be implemented
    - E.g., castration, ovariectomy, laparotomy, jugular catheter placement, craniotomy +/- implant, spared nerve injury, thoracotomy
    - Where surgery is involved, a local anesthetic should be included in the protocol
    - These three classes of analgesics (NSAIDS, opioids, local) can be safely combined
- Anesthesia refers to the loss of consciousness and sensation but does not necessarily entail the loss of sensitivity to pain - analgesia should always be provided with anesthesia
- Preventative analgesia, where pain control is provided before pain circuits are activated vs. after observing clinical signs of pain, is strongly preferred for both humane and scientific reasons:
    - Providing analgesia before a painful procedure and re-dosing at appropriate intervals reduces the intensity of the painful stimulus, which decreases the amount of anesthetic agent required to maintain a surgical plane of anesthesia (in turn decreasing the risk of an anesthetic overdose), and smooths recovery
    - Analgesia should be provided immediately after animals are anesthetized for surgery, i.e., before shaving, surgical prep etc., to maximize the time between administration and the first incision. Analgesia can also be provided prior to anesthesia if the animal is positively habituated to handling
- After a procedure, animals should be monitored using validated species-specific indicators of pain so that follow-up analgesia can be provided if necessary
- Rats and mice who are painful are at risk of becoming dehydrated. Animals placed under general anesthesia for a painful procedure should receive a rehydration injection of warmed sterile, isotonic fluids (e.g., 0.9% sodium chloride) while anesthetized
    - 20 mL/kg is an appropriate starting point; consult with a veterinarian if multiple anesthesia events will occur within a week or if the procedure carries a high risk of blood loss
- Consideration should be given to attempting a non-invasive administration method, especially in animals that are handled routinely and habituated to syringe feeding
- Research into ideal dosing and administration of analgesics for rodents is ongoing and recommendations may change
- Analgesic requirements for different predicted levels of pain can be found in Appendix 4; changes must be justified to the ACC

CLASSES OF ANALGESICS

NSAIDS

NSAIDS provide modest pain control and are useful for controlling mild pain but must be combined with other analgesics for controlling moderate to severe pain. They can additionally reduce inflammation and fever. NSAIDS are not controlled substances, but they can cause gastric upset or kidney/liver disease and can reduce clotting ability. These side effects are more likely with increasing length of usage; unless directed otherwise by a veterinarian, NSAID administration should be limited to three days. In the context of surgery, NSAIDs are ideally administered after the procedure, if a different analgesic can be used pre-operatively (such as an opioid) to prevent activation of pain circuits.

Carprofen and meloxicam are the most used NSAIDS in laboratory rodents. Injectable meloxicam can be diluted for accurate dosing of smaller animals and is also available in an oral formulation. The context of administration (e.g., type of procedure, animal's health status, severity of pain) will determine formulation and route.

An overview of the details of administration of the different formulations of carprofen and meloxicam can be found in Table A1.1. Example doses can be found in Tables A1.2 and A1.3.

Dilution information (see Appendix 5 for detailed instructions)

For all rodents, carprofen must be diluted to allow for accurate dosing. For rats <160 g and mice <45 g, meloxicam must also be diluted.

OPIOIDS

Opioids provide potent pain control and are therefore a vital part of a multi-modal analgesia regimen for managing moderate to severe pain. As all opioids are controlled substances, their use for any purpose requires special record keeping. Unlike NSAIDS, opioids pose no additional risk of bleeding or organ injury under anesthesia and are the ideal pre-operative analgesic. Opioids may cause mild respiratory depression, sedation, and slower recovery from anesthesia; however, the improved comfort of the animal generally outweighs these side effects. The antinociceptive effects of opioids will allow animals to be maintained at a surgical plane of anesthesia with a lower percentage of inhalant compared to using a pre-operative NSAID or no analgesia.

Occasionally, opioids may cause hyperactivity and ingestion of bedding or self-mutilation (particularly in rats), aka pica. Under the supervision of a veterinarian, using a lower dose and providing appropriate enrichment will resolve these issues in most cases.

Buprenorphine is the most used opioid for laboratory rodents. Buprenorphine is available in both a standard (0.3 mg/mL) and sustained release (SR) formulation (compounded formulation 0.6 mg/mL; other formulations exist). The standard 0.3 mg/mL formulation can be administered via multiple routes and can also be diluted for accurate dosing of smaller animals. The context of administration (e.g., type of procedure, animal's health status, severity of pain) will determine formulation and route.

An overview of the details of administration and procurement of the different formulations of buprenorphine can be found in Table A2.1. Example doses can be found in Tables A2.2 and A2.3, and instructions for reversal with naloxone in the case of opioid overdose can be found in Table A2.4.

Unique details of SQ administration of SR buprenorphine (0.6 mg/mL):

- When dosing multiple animals, transfer entire daily volume to a multi-use vial and allow to come to room temperature prior to drawing up small individual volumes (the drug is stable for 72 hours outside of the fridge)
- Draw up using an 18-gauge needle, as the drug is viscous, then switch to a new, appropriately sized needle (i.e., 23-27) for injection
    - To avoid drug loss in the needle and syringe hub, draw back the plunger and pull the entire volume of drug into the syringe prior to removing the first needle.
- Administer subcutaneously between the shoulder blades, injecting SLOWLY
- Continue pinching the injection site for 10-15 seconds after removing the needle (if anesthetized) or as long as the animal does not struggle (if awake)
- Whenever possible, perform the injection under anesthesia
- Store extra SR buprenorphine in the fridge
- Animals are likely to demonstrate signs of sedation (ataxia, sleeping when undisturbed) with this formulation – this is normal. However, if animals are difficult to arouse or are not eating/drinking within 3 hours of surgery, consult a veterinarian to discuss a dose adjustment

Dilution information (see Appendix 5 for detailed instructions)

For rats that are less than 350 g, regular buprenorphine must be diluted to allow for accurate dosing. For rats that are equal to or greater than 350 g, either the undiluted stock solution or the diluted solution may be used, depending on the dose chosen and the user's comfort with drawing up and injecting small volumes.

For mice, regular buprenorphine must be diluted to administer doses at the lower end of the dose range. The threshold for which to switch from diluted to undiluted depends on the weight of the

mouse, to balance accurate dosing while staying within appropriate injection volume limits.

LOCAL ANESTHETICS

Local anesthetics provide pain control by blocking nerve conduction at the site of administration and are an important part of a surgical analgesic regimen. They should be administered pre-operatively at the site of a procedure before the initial incision but can also be used for managing severe post-operative pain.

Lidocaine and bupivacaine are the most used local anesthetics for laboratory rodents. They are injected subcutaneously and should be diluted for accurate dosing. Rarely, local anesthetics may be applied topically for transdermal absorption as a precautionary measure (e.g., prior to ear notching or after tail snipping), but the length of time required for effect and the risk of removal by the animal precludes topical use in most cases.

An overview of the details of administration and procurement of the different formulations of lidocaine and bupivacaine can be found in Table A3.1. Example doses can be found in Tables A3.2.

Dilution information (see Appendix 5 for detailed instructions)

For all rodents, lidocaine and bupivacaine must be diluted to allow for accurate dosing.

ADJUNCTIVE DRUGS

Research into the use of drugs that provide a sedative/anxiolytic/tranquilizing effect alongside traditional analgesics in rodents is ongoing. These drugs include trazodone, gabapentin, acepromazine, and midazolam. Consult with your facility veterinarian if you are interested in using one of these drugs.

VOLUNTARY ORAL ADMINISTRATION OF ANALGESICS

General principles

- Animals should be habituated to syringe feeding and, if using a vehicle, palatability confirmed prior to attempting voluntary oral administration
    - I.e., ensure animals will readily consume the appropriate volume of something palatable from a syringe
- Aim for the smallest volume that can be dosed accurately – drugs that are diluted for injectable use may be left at full strength for oral administration, as the dose is usually

higher
    - Micropipettes are useful for dosing small volumes in mice
- Administer the medication into the mouth via a syringe one small drop at a time while the animal swallows - forcefully injecting the entire dose at once can startle the animal or cause it to aspirate
- Commercial food tablets/treats containing medications or gels that can be mixed with medications are also available from companies such as Bio-Serv and Clear H2O
- The ideal vehicle varies based on individual preference and drug volume, but something sweet is ideal e.g., sugar water, condensed milk, Nutella, icing, Jell-O, maple syrup, strawberry milkshake
    - The ideal medication: vehicle ratio varies depending on the bitterness and volume of the drug, but a useful starting point is 1 part drug to 2-3 parts vehicle
- Example recipe – this mixture is sweet enough to disguise the taste of medication but runny enough to mix:
    - Mix 1 part sucrose with 2 parts boiling water to create a 50% sucrose solution (e.g., 50 g sucrose with 100 mL water)
    - Mix 1 part sweetened condensed milk with 3 parts 50% sucrose solution (e.g., 1 tablespoon condensed milk with 3 tablespoons sucrose solution)
    - Stir or shake vigorously
    - Allow to cool before using; large volumes can be kept in the freezer for long-term storage

NSAIDS

- For cases of mild pain where a large group of mice must be dosed, carprofen can be administered via drinking water
    - Consult with a facility staff member for preparation of carprofen water for mice (not rats)
- Mice and rats **may** consume oral carprofen, if mixed with a palatable vehicle and animals are habituated to syringe feeding
- Rats will sometimes consume plain oral meloxicam readily if habituated to syringe feeding, but may require a palatable vehicle
- Mice may consume oral meloxicam without a vehicle, if habituated to syringe feeding, but will likely require a palatable vehicle

Buprenorphine

- Both rats and mice will generally consume buprenorphine with a vehicle (see example recipe), although higher doses (assuming a 1:3 drug to vehicle ratio) may exceed the volume animals will readily consume in a single feeding bout

References

CCAC Guidelines: Mice
https://www.ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Mice.pdf

CCAC Guidelines: Rats
https://ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Rats.pdf

Curtin, L. I., Grakowsky, J. A., Suarez, M., Thompson, A. C., DiPirro, J. M., Martin, L. B., & Kristal, M. B. (2009). Evaluation of Buprenorphine in a Postoperative Pain Model in Rats. Comparative Medicine, 59(1), 60–71.

Foley, P. L., Kendall, L. V., & Turner, P. V. (2019). Clinical Management of Pain in Rodents. Comparative Medicine, 69(6), 468–489. https://doi.org/10.30802/AALAS-CM-19-000048

UBC Animal Care Committee: Analgesia for Adult Mice and Rats - Buprenorphine SOP
https://animalcare.ubc.ca/sites/default/files/documents/Opioid%20Buprenorphine%20Analgesia%20for%20Adult%20Mice%20and%20Rats%20SOP%20June%202020%20FIN....pdf

UBC Animal Care Committee: Rodent Procedures Classifications and Analgesia Requirements
https://animalcare.ubc.ca/sites/default/files/documents/Guideline-Surgical%20Class%20and%20Analgesia%20Guidelines%20%282016%29.pdf

Appendix 1 – NSAIDS- SOP.ACS.814 Administration of pain control to rats and mice 2023

Table A1.1. Details of administration of carprofen and meloxicam

Formulation	Dose (mg/kg)	Frequency	Stock concentration (mg/mL)	Dilution (if necessary, mg/mL)	Route(s)	Refrigeration required?
Carprofen	Mice: 20 on first day, then 10 on following days Rats: 5	Every 24 hrs	50	5	SQ, oral in water[+]	Yes
Meloxicam – injectable	Mice: 5 Rats: 1-2[*] on first day, then 1 on following days	Every 24 hrs	5	0.5 (for rats <160 g and mice <45 g)	SQ	No
Meloxicam – oral	Mice: 5 Rats: 1-2[*] on first day, then 1 on following days	Every 24 hrs	1.5	N/A	Voluntary oral	No

[*]Risk of side effects increases with higher doses – consider using multimodal analgesia to allow effective use of lower doses

[+]Consult facility staff/veterinarian for preparation of carprofen water. May only be used for managing mild pain in large groups, e.g., after identification/genotyping procedures

Table A1.2. Example dose volumes of carprofen and meloxicam for rats using different dilutions and routes

Weight (g)	Carprofen diluted (5 mg/mL) Injectable use	Meloxicam undiluted (5 mg/mL) Injectable use	Meloxicam diluted (0.5 mg/mL) Injectable use	Meloxicam undiluted (1.5 mg/mL) Oral use
150	0.15 mL	N/A – use diluted	0.3 – 0.6 mL	0.1 – 0.2 mL
250	0.25 mL	0.05 – 0.1 mL	0.5 – 1.0 mL	0.17 – 0.33 mL
350	0.35 mL	0.07 – 0.14 mL	0.7 – 1.4[+] mL	0.23 – 0.46 mL
450	0.45 mL	0.09 – 0.18 mL	0.9 – 1.8[+] mL	0.3 – 0.6 mL
550	0.55 mL	0.11 – 0.22 mL	1.1 – 2.2[+] mL	0.37 – 0.73 mL

[+]Consider using undiluted solution for upper end of dose range

Table A1.3. Example dose volumes of carprofen for mice using different dilutions and routes

Weight (g)	Carprofen diluted (5 mg/mL) Injectable use	Meloxicam undiluted (5 mg/mL) Injectable use	Meloxicam diluted (0.5 mg/mL) Injectable use	Meloxicam undiluted (1.5 mg/mL) Oral use
15	0.03 – 0.06 mL	N/A – use diluted	0.15 mL	0.05 mL
25	0.05 – 0.1 mL	N/A – use diluted	0.25 mL	0.08 mL
35	0.07 – 0.14 mL	N/A – use diluted	0.35 mL	0.12 mL
45	0.09 – 0.18 mL	0.05 mL	0.45 mL	0.15 mL
55	0.11 – 0.22 mL	0.06 mL	0.55 mL	0.18 mL

Appendix 2 – Opioids - SOP.ACS.814 Administration of pain control to rats and mice 2023

Table A2.1. Details of administration and procurement of standard and sustained-release formulations of buprenorphine

Formulation	Dose (mg/kg)	Frequency	Stock concentration (mg/mL)	Dilution (if necessary, mg/mL)	Routes	Refrigeration required?	Health Canada exemption	Dispensing procedure to research team
Buprenorphine	Mice: 0.1-0.5 (injectable) or 1 (oral) Rats: 0.05 – 0.1(injectable) or 0.4 (oral)	Every 6-8 hrs	0.3	0.03	SQ*, IP*, voluntary oral	No	Required for planned experimental use.	Provided in bulk as per Health Canada exemption for experimental use, or on an as-needed basis for veterinary recommended treatment
Sustained-Release Buprenorphine	Mice: 0.5-1.0 Rats: 0.6-1.2	Every 48 hrs	0.6 - most commonly	N/A	SQ* only	Yes long-term, but stable for 72 hours at room temperature	Not required at this time.	Provided daily on an as-needed basis from the CAF (or weekly if the research team has access to a double locked refrigerator)

Table A2.2. Example dose volumes of buprenorphine for rats using different formulations, dilutions, and routes

Weight (g)	Buprenorphine undiluted (0.3 mg/mL) Injectable use for animals ≥ 350 g	Buprenorphine diluted (0.03 mg/mL) Injectable use	Buprenorphine undiluted (0.3 mg/mL) Oral use	SR Buprenorphine undiluted (0.6 mg/mL) Injectable use
150	N/A – use diluted	0.25 – 0.5 mL	0.20 mL	0.15- 0.30* mL
250	N/A – use diluted	0.42 – 0.83 mL	0.33 mL	0.25 - 0.50* mL
350	0.04 – 0.12 mL	0.58 – 1.12+ mL	0.47 mL	0.35 - 0.70* mL
450	0.05 – 0.15 mL	0.75 – 1.5+ mL	0.60 mL	0.45 - 0.90* mL
550	0.06 – 0.18 mL	0.92 – 1.83+ mL	0.73 mL	0.55 - 1.1* mL

+Consider using undiluted solution for upper end of dose range

*Start at lower end of dose range and increase if adequate analgesia is not achieved

Appendix 2 – Opioids *(continued)* - SOP.ACS.814 Administration of pain control to rats and mice 2023

Table A2.3. Example dose volumes of buprenorphine for mice using different formulations, dilutions, and routes

Weight (g)	Buprenorphine undiluted (0.3 mg/mL) Injectable use for mid-high dose*	Buprenorphine diluted (0.03 mg/mL) Injectable use for low-mid dose*	Buprenorphine undiluted (0.3 mg/mL) Oral use	SR Buprenorphine undiluted (0.6 mg/mL) Injectable use**
15	0.5 mg/kg: 0.03 mL	0.1 – 0.4 mg/kg: 0.05 – 0.2 mL	0.05 mL	0.01 – 0.03 mL
25	0.5 mg/kg: 0.04 mL	0.1– 0.4 mg/kg: 0.08 – 0.33 mL	0.08 mL	0.02 - 0.04 mL
35	0.4 – 0.5 mg/kg: 0.05 mL – 0.06 mL	0.1 – 0.3 mg/kg: 0.12 – 0.35 mL	0.12 mL	0.03 - 0.06 mL
45	0.3 – 0.5 mg/kg: 0.05 – 0.08 mL	0.1 – 0.2 mg/kg: 0.15 – 0.3 mL	0.15 mL	0.04 - 0.08 mL
55	0.03 – 0.05 mg/kg: 0.05 – 0.09 mL	0.1 – 0.2 mg/kg: 0.18 – 0.37 mL	0.18 mL	0.05 - 0.09 mL

*In most cases, start with 0.3 mg/kg dosing and adjust based on pain assessments. Minor pain may only require 0.1 mg/kg, while severe pain may require up to 0.5 mg/kg
**Start at 1.0 mg/kg for mice with a C57Bl/6 background and 0.5 mg/kg for all other strains. Adjust based on pain assessments. Contact ACS veterinarians for use with DBA mice.

Table A2.4. Emergency instructions for treating opioid overdose (dangerous respiratory suppression; excessive sedation) with 0.4 mg/mL naloxone

	Mice	Rats
Dilution	Mix 0.1 mL naloxone with 4.9 mL sterile saline	Mix 0.2 mL naloxone with 4.8 mL sterile saline
Volume to administer	0.05 mL for small – medium mice 0.07 mL for large mice	1.2 mL for small – medium rats 1.3 mL for large rats
Route	IV, IP, or SQ	
Instructions	*Titrate to effect with repeated doses (ideally under veterinary supervision) but wait at least 10 minutes between doses to observe effects for SQ/IP route and 1 minute for IV route. If the opioid was used to manage pain, the goal is partial, not full, reversal: as soon as dangerous respiratory depression or excessive sedation begin to resolve, discontinue administration. For longer acting opioids, multiple doses may be required, as naloxone has a short duration of effect (~30-40 minutes in companion animal species).*	

Appendix 3 – Local Anaesthetics - SOP.ACS.814 Administration of pain control to rats and mice 2023

Table A3.1. Details of administration of lidocaine and bupivacaine

Drug	Maximum dose (mg/kg)	Time to effect	Duration of effect	Stock concentration (mg/mL)	Dilution (mg/mL)	Route(s)	Refrigeration required?
Lidocaine neat (No epinephrine)	7	<5 mins	<1 hours	20 (2%)	5 (0.5%)	SQ; transdermal	No
Bupivacaine	8	20 mins	4-8 hours	5 (0.5%)	2.5 (0.25%)	SQ; transdermal	No

Table A3.2. **Maximum**[+] dose volumes of subcutaneous lidocaine and bupivacaine for rats and mice

Rats		
Weight (g)	**Lidocaine (0.5%)**	**Bupivacaine (0.25%)**
150	0.21 mL	0.48 mL
250	0.35 mL	0.80 mL
350	0.49 mL	1.12 mL
450	0.63 mL	1.44 mL
550	0.77 mL	1.76 mL
Mice		
Weight (g)	**Lidocaine (0.5%)**	**Bupivacaine (0.25%)**
15	0.02 mL[*]	0.05 mL
25	0.03 mL[*]	0.08 mL
35	0.05 mL	0.11 mL
45	0.06 mL	0.14 mL
55	0.07 mL	0.17 mL

[+] **Do not exceed.** Dose can be divided if multiple incisions are planned, and a lower volume can be used for a small incision.

[*]Consider diluting further to 0.25% to get larger volumes for entire length of incision

Appendix 4 – Standard analgesia and monitoring requirements for rodent procedures – SOP.ACS.814 Administration of pain control to rats and mice 2023

Analgesia requirements		
	Minor pain[+]	**Moderate – severe pain**[+]
Example procedures	• Subcutaneous implant • Intramuscular injection • Cancer cell injection • Tracheal injection • Skin biopsy	• Castration • Ovariectomy • Laparotomy • Thoracotomy • Jugular catheter placement • Craniotomy +/- implant • Spared nerve injury
Standard pre-procedural analgesic regimen	• Buprenorphine[*] (SR or regular depending on procedure) OR NSAID • +/- Lidocaine or bupivacaine (for surgery, 5 or10 mins pre incision at planned site, respectively)	• SR buprenorphine[*] (immediately after induction of anaesthesia) AND • Bupivacaine (10 mins pre incision at planned site)
Standard post-procedural Analgesic regimen	• +/- Buprenorphine[*] OR NSAID if painful on assessment	• NSAID (immediately after procedure, while under anaesthesia) at loading dose AND • NSAID again at 24 and 48 hours at maintenance dose • +/- SR buprenorphine at 48 hours if painful on assessment
Total # of days (including day 0)	1	3 – 5
Monitoring requirements		
	Minor pain	**Moderate – severe pain**
# Total # of days (including day 0)	2	5 – 7
# Of times/day	1 – 2	2 – 3

[+]Any procedure may warrant reclassification into a higher category by the ACC based on the severity of the anticipated pain level, which can vary according to skill of surgeon.

[*]Or other appropriate opioid, administered at suitable intervals

Appendix 5 - Dilution instructions and drug labels- SOP.ACS.814 Administration of pain control to rats and mice 2023

Table A.5.1: Drug dilution instructions and example calculations

Calculation steps	General formula	Carprofen (for 10 mL of solution)	Meloxicam (for 10 mL of solution)	Buprenorphine (for 10 mL of solution)	Lidocaine (for 4 mL of solution)	Bupivacaine (for 4 mL of solution)
Step 1: confirm stock and desired concentration	Mg/mL stock to mg/mL dilution	50 mg mL to 5 mg/mL	5 mg/mL to 0.5 mg/mL	0.3 mg/mL to 0.03 mg/mL	20 mg/mL to 5 mg/mL*	5 mg/mL to 2.5 mg/mL
Step 2: calculate volume of stock solution	**mL stock =** $\frac{\text{concentration required (mg/mL)}}{\text{stock concentration (mg/mL)}}$ * volume required (mL)	= (5 / 50) * 10 **= 1 mL stock**	= (0.5 / 5) * 10 **= 1 mL stock**	= (0.03 / 0.3) * 10 **= 1 mL stock**	= (5 / 20) * 4 **= 1 mL stock**	= (2.5 / 5) * 4 **= 2 mL stock**
Step 3: calculate volume of sterile diluent	**mL dilutant =** volume required (mL) – volume of stock (mL)	= 10 – 1 **= 9 mL saline**	= 10 – 1 **= 9 mL saline**	= 10 – 1 **= 9 mL saline**	= 4 - 1 **= 3 mL saline**	= 4 - 2 **= 2 mL saline**
Step 4: confirm dilution concentration	**mg/mL dilution =** $\frac{\text{Volume stock (mL) * concentration stock (mg/mL)}}{\text{Total dilution volume (mL)}}$	= (1 * 50) / 10 **= 5 mg/mL**	= (1 * 5) / 10 **= 0.5 mg/mL**	= (1 * 0.3) / 10 **= 0.03 mg/mL**	= (1*20) / 4 **= 5 mg/mL**	= (2 * 5) / 4 **= 2.5 mg/mL**
Step 5: LABEL AND STORE IN A GLASS, AMBER, MULTI-USE VIAL - PROTECT FROM LIGHT Label contents: drug name, concentration, stock concentration, volume prepared, date of preparation, discard date (usually 30 days), initial of preparer, additional storage instructions Protect label with clear tape or use a heat fixed label						

*Or 2.5 mg/ml for very small mice, see Table A3.2

Table A.5.2: Animal weight thresholds for diluting injectable analgesics

Species	Carprofen	Meloxicam	Buprenorphine	Lidocaine	Bupivacaine
Rats	Always dilute	<160 g	<350 g	Always dilute	Always dilute
Mice	Always dilute	<45 g	Dose dependent – see Table A.2.3	Always dilute (two possible strengths)	Always dilute

Figure A.5.1. Example drug dilution label.