STANDARD OPERATING PROCEDURE #101

RODENT ANALGESIA

1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe methods of assessing pain in rodents and mitigating pain by administration of analgesic medications.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinary care staff.

3. GENERAL CONSIDERATIONS

3.1. A procedure which would be expected to be painful if it were done on humans must be considered painful to the animal.

3.2. When there is a question of whether or not a procedure is painful, the animal should receive the benefit of analgesia.

3.3. Analgesia should be provided at an appropriate dose and frequency to control pain.

3.4. Any deviation from this procedure must be justified by the investigator and approved by the appropriate Facility Animal Care Committee (FACC).

4. PAIN RECOGNITION AND ASSESSMENT

4.1. Adapt the frequency of observation to the protocol (minimum once a day).

4.2. Start by observing the animal from a distance so the animal's behavior is not altered by the presence of the observer. Then proceed to observe the animal more closely.

4.3. Look for any changes in the behavior. Report animals which appear to be in pain to the veterinary care staff.

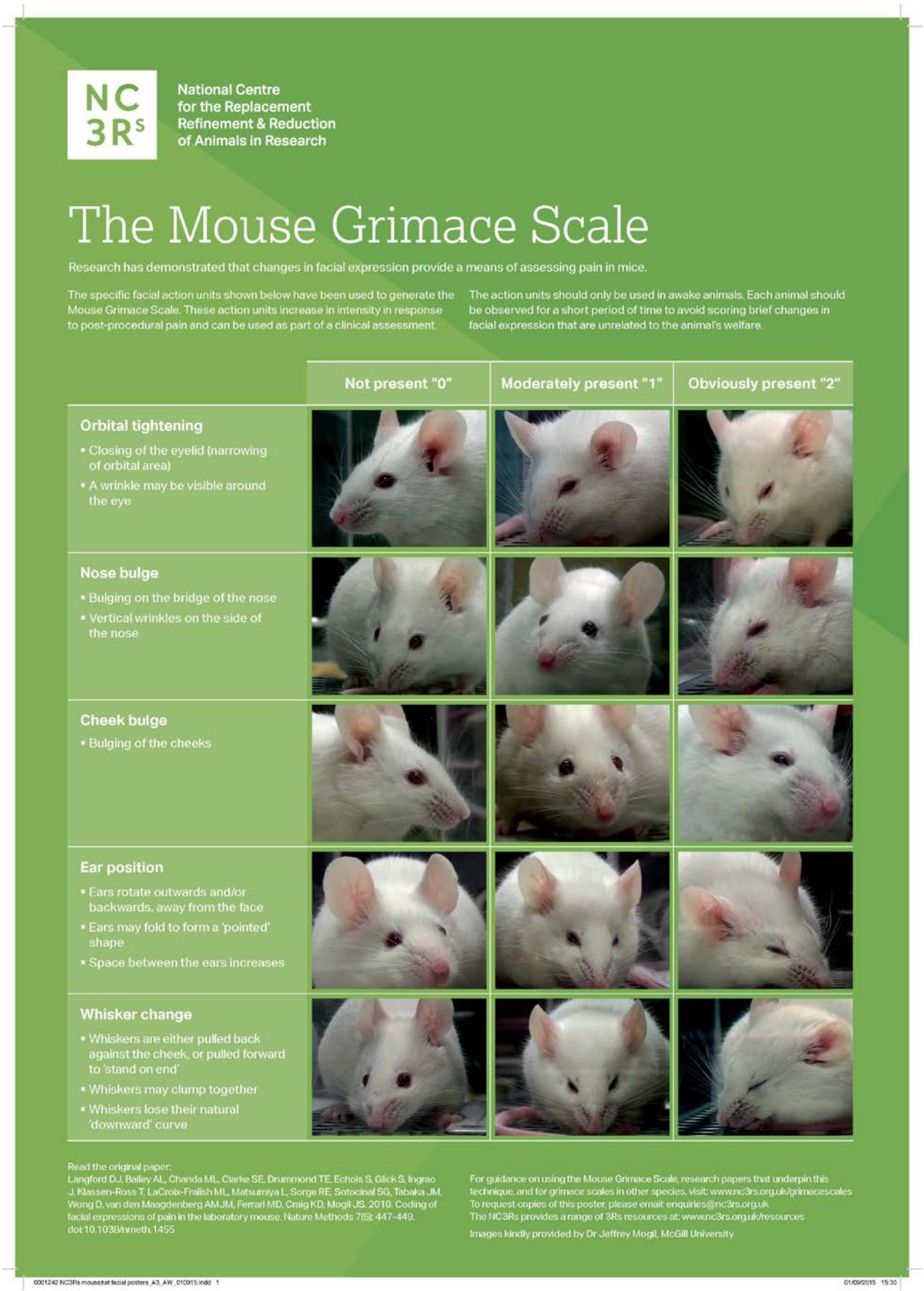
4.4. Common clinical signs indicative of pain or distress include:

- 4.4.1. Avoidance
- 4.4.2. Vocalization
- 4.4.3. Aggressiveness (mainly if the animal cannot escape)
- 4.4.4. Spontaneous activities are reduced. The animal is isolated from the social group
- 4.4.5. Altered gait
- 4.4.6. Hunched posture
- 4.4.7. Piloerection
- 4.4.8. Reduced grooming; dark-red stain around the eyes and nostrils
- 4.4.9. Reduced appetite and subsequent weight loss

Note: The most reliable signs of pain and distress are the changes in behavior. This implies a good knowledge of species and individual normal behavior by the observer.

4.5. The Mouse Grimace Scale (Langford et al. 2010):

The mouse grimace scale is a standardized behavioral coding system that demonstrates facial expressions which can be used to assess pain in the laboratory mouse.



NC 3Rs

National Centre for the Replacement Refinement & Reduction of Animals in Research

The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening ▪ Closing of the eyelid (narrowing of orbital area) ▪ A wrinkle may be visible around the eye			
Nose bulge ▪ Bulging on the bridge of the nose ▪ Vertical wrinkles on the side of the nose			
Cheek bulge ▪ Bulging of the cheeks			
Ear position ▪ Ears rotate outwards and/or backwards, away from the face ▪ Ears may fold to form a 'pointed' shape ▪ Space between the ears increases			
Whisker change ▪ Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' ▪ Whiskers may clump together ▪ Whiskers lose their natural 'downward' curve			

Read the original paper:
Langford D.J, Bailey A.L, Chanda M.L, Clarke S.E, Drummond T.E, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish M.L, Matsumiya L, Sorge R.E, Sotocinal S.G, Tabaka J.M, Wong D, van den Maagdenberg A.M.J.M, Ferrari M.D, Craig K.D, Mogil J.S. 2010. Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7(6): 447-449. doi:10.1038/nmeth.1455

For guidance on using the Mouse Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimacescales
To request copies of this poster, please email: enquiries@nc3rs.org.uk
The NC3Rs provides a range of 3Rs resources at: www.nc3rs.org.uk/resources

Images kindly provided by Dr Jeffrey Mogil, McGill University

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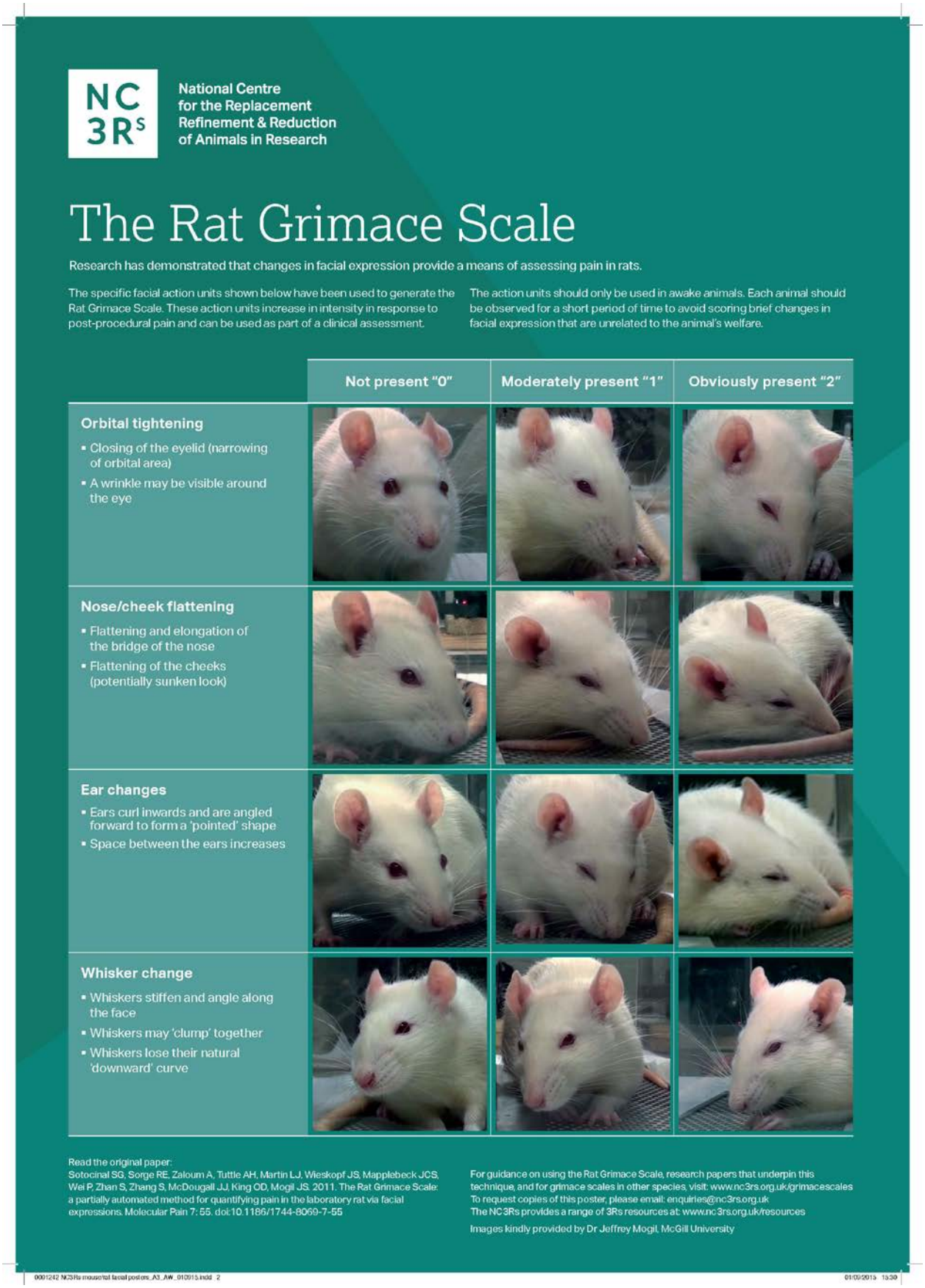
4.6. The Rat Grimace Scale (Sotocinal et al. 2011):

The rat grimace scale is a standardized behavioral coding system that demonstrates facial expressions which can be used to assess pain in the laboratory rat.



NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

The Rat Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rats.

The specific facial action units shown below have been used to generate the Rat Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening ▪ Closing of the eyelid (narrowing of orbital area) ▪ A wrinkle may be visible around the eye			
Nose/cheek flattening ▪ Flattening and elongation of the bridge of the nose ▪ Flattening of the cheeks (potentially sunken look)			
Ear changes ▪ Ears curl inwards and are angled forward to form a 'pointed' shape ▪ Space between the ears increases			
Whisker change ▪ Whiskers stiffen and angle along the face ▪ Whiskers may 'clump' together ▪ Whiskers lose their natural 'downward' curve			

Read the original paper:
Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JCS, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Molecular Pain 7: 55. doi:10.1186/1744-8069-7-55

For guidance on using the Rat Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimacescales
To request copies of this poster, please email: enquiries@nc3rs.org.uk
The NC3Rs provides a range of 3Rs resources at: www.nc3rs.org.uk/resources

Images kindly provided by Dr Jeffrey Mogil, McGill University

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5. ANALGESIA PLAN

5.1. If possible, provide analgesia before the painful stimulus, as it is more effective in preventing pain (e.g. give analgesic before surgery).

5.2. Use a combination of analgesics, which is often more effective than using a single agent.
For example, administer a combination of buprenorphine, carprofen, and local infiltration of lidocaine/bupivacaine.

5.3. For surgical procedures, extend analgesia from pre-op to 72 hours post-op, unless specified otherwise in the Animal Use protocol (AUP) and approved by the FACC.

6. LOCAL ANALGESIA

6.1. Infiltrate or apply local analgesic to areas where a painful stimulus may be induced. Repeat application of local agent at specified intervals to maintain analgesia. In some cases a sedative is recommended when using local analgesia.

Analgesic	Dose	Duration	Note
Lidocaine	< 2 mg/kg	30–60 min.	Use lidocaine HCl 2% (20mg/ml) injectable solution. Because this drug is acidic, it is recommended to dilute it 3:1 with sodium bicarbonate injectable solution (at 5 or 8.4%). Dilution with sodium bicarbonate is not necessary if lidocaine is to be administered to an anesthetized animal. Dilution must be prepared immediately before use and should not be stored. Diluted solution is as effective but induction of analgesia is slightly prolonged.
Bupivacaine	< 2 mg/kg	3–4 hrs.	Use bupivacaine HCl 0.50% (5mg/ml) injectable solution. Same comment as for lidocaine.
* Lidocaine-bupivacaine mixture	-	30min. to 4 hrs.	Same comment as for lidocaine. Combining both drugs allows for rapid induction and prolonged effect. Discard mixture after 3 months.
EMLA cream	Thick spread	30–60 min.	Shave or pluck the fur and apply a thick layer of cream ideally 10 minutes before the painful procedure.

*most commonly used

7. GENERAL ANALGESIA

Mouse / Hamster / Gerbil

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine Slow Release (SR)	1 mg/kg	SC	once	Buprenorphine SR (0.6mg/ml) is a sustained release buprenorphine product that has been developed to provide 72 hours of analgesia and is specifically designed for use in rodents. See administration instructions in section 7.1.
*Buprenorphine	0.1 mg/kg	SC, IP	4–8 hrs.	Mild to moderate pain. Controlled drug.
*Carprofen	20 mg/kg	SC, PO	12–24 hrs.	Mild to moderate pain. Use carprofen 50mg/ml injectable solution. To prepare a 4mg/ml dilution: add 0.8ml of carprofen 50mg/ml to 9.2 ml of sterile water for injection. Administer 5µL/g of bodyweight. Discard dilution after 3 months.
*Meloxicam	20 mg/kg	SC, PO	24 hrs.	Mild to moderate pain.
Ketoprofen	15 mg/kg	SC	12-24 hrs.	Mild to moderate pain. Do not administer after an adrenalectomy.
Acetaminophen	300 mg/kg 2 mg/ml solution in drinking water	PO	Give medicated water 24 hours before painful procedure.	Mild pain; to combine with another analgesic. Used in conjunction with buprenorphine enhances pain relief. Provide acetaminophen-treated drinking water at least 24 hours in advance of painful procedure. Prepare solution by mixing Children's Tylenol suspension liquid to drinking water. Mix bottle thoroughly before use. Fresh solution should be prepared and added to clean bottles every 3-4 days for maximum efficacy.
Fentanyl	0.1 mg/kg	SC, IP	30 min.	Moderate to severe pain. Controlled drug.
Morphine	20 mg/kg	SC, IP	2 hrs.	Moderate to severe pain. Controlled drug.

*most commonly used

Rat

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine Slow Release (SR)	1 mg/kg	SC	once	Buprenorphine SR (0.6mg/ml) is a sustained release buprenorphine product that has been developed to provide 72 hours of analgesia and is specifically designed for use in rodents. See administration instructions in section 7.2. Controlled drug.
*Buprenorphine	0.05 mg/kg	SC, IP, IV	8-12 hrs.	Mild to moderate pain. Controlled drug. Give every 12 hours when combined with meloxicam or carprofen.
*Carprofen	5-10 mg/kg	SC, PO	12–24 hrs.	Mild to moderate pain. Use carprofen 50mg/ml injectable solution. To prepare a 5mg/ml dilution: add 1ml of carprofen 50mg/ml to 9 ml of sterile water for injection. Administer 0.1-0.2ml/100g of bodyweight. Discard dilution after 3 months. Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.
*Meloxicam	1 mg/kg	SC, PO	24 hrs.	Mild to moderate pain.
Ketoprofen	2–5 mg/kg	SC, PO	12–24 hrs.	Mild to moderate pain. Do not administer after an adrenalectomy.
Acetaminophen	100–300 mg/kg 5 mg/ml solution in drinking water	PO	Give medicated water 24 hours before painful procedure.	Mild pain; to combine with another analgesic. Used in conjunction with buprenorphine enhances pain relief. Provide acetaminophen-treated drinking water at least 24 hours in advance of painful procedure. Prepare solution by mixing Children's Tylenol suspension liquid to drinking water. Mix bottle thoroughly before use. Fresh solution should be prepared and added to clean bottles every 3-4 days for maximum efficacy.
Fentanyl	0.1 mg/kg	SC, IP	30 min.	Moderate to severe pain. Controlled drug.
Morphine	20 mg/kg	SC, IP	2 hrs.	Moderate to severe pain. Controlled drug.

*most commonly used

Guinea pig / Chinchilla

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine	0.01–0.05 mg/kg	SC, IP	8–12 hrs.	Mild to moderate pain. Controlled drug.
*Carprofen	5-10 mg/kg	SC, PO	12–24 hrs.	Mild to moderate pain. Use carprofen 50mg/ml injectable solution. To prepare a 5mg/ml dilution: add 1ml of carprofen 50mg/ml to 9 ml of sterile water for injection. Administer 0.1-0.2ml/100g of bodyweight. Discard dilution after 3 months. Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.
*Meloxicam	1 mg/kg	SC, PO	24 hrs.	Mild to moderate pain.
Ketoprofen	2 mg/kg	SC	12–24 hrs.	Mild pain. Do not administer after an adrenalectomy.
Acetaminophen	100–300 mg/kg	PO	Give medicated water 24 hours before painful procedure.	Mild pain; to combine with another analgesic. Used in conjunction with buprenorphine enhances pain relief. Provide acetaminophen-treated drinking water at least 24 hours in advance of painful procedure. Prepare solution by mixing Children's Tylenol suspension liquid to drinking water. Mix bottle thoroughly before use. Fresh solution should be prepared and added to clean bottles every 3-4 days for maximum efficacy.

*most commonly used

7.1. Administration of non-steroidal anti-inflammatory drugs (NSAIDs):

7.1.1. NSAIDs include carprofen, ketoprofen and meloxicam.

7.1.2. Ensure good water intake and monitor hydration status during the treatment period.

7.1.3. Suspend water restriction prior to administration of NSAIDs.

7.1.4. To minimize chances for adverse drug interactions, a washout period of 5-7 days is recommended before switching between NSAIDs.

7.2. Administration instructions for buprenorphine slow release (SR):

7.2.1. Avoid contact with the skin to prevent the development of injection site reactions:

7.2.2. For use in mice: draw up the buprenorphine SR with a 25g or 27g needle attached to a 1cc low waste syringe (alternatively, use a 28g insulin syringe) and wipe needle off with an alcohol swab to remove any drug residue from the needle prior to administration.

7.2.3. For use in rats: draw up the buprenorphine SR with an 18g needle and change to a 23g needle prior to administration

7.2.4. Administer subcutaneously on back of neck between the shoulder blades while the animal is anesthetized.

7.2.5. Administer slowly and finish injecting before the needle is pulled out through skin.

7.2.6. Pinch the injection site for approximately 10 seconds after removing the needle

7.2.7. Do not combine the buprenorphine SR with any other drugs in the same syringe and do not attempt to dilute the formulation.

8. REFERENCES

8.1. Dickinson AL, Leach MC, Flecknell PA. (2009). The analgesic effects of oral paracetamol in two strains of mice undergoing vasectomy. *Lab Anim* **43**:357-61.

8.2. Fernández-Dueñas V, Poveda R, Sánchez S, Ciruela F.(2012). Synergistic interaction between fentanyl and a tramadol: paracetamol combination on the inhibition of nociception in mice. *J Pharmacol Sci*. **118**:299-302.

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8.4. Garrone B, Polenzani L, De Santi S, Moreci W, Guglielmotti A (2007). Paracetamol reduces neuropathic pain-like behavior in rats by potentiating serotonergic neurotransmission. *IJIB* **1**: 197-205.

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8.10. Mouedden ME, Meert TF. (2007). Pharmacological evaluation of opioid and non-opioid analgesics in a murine bone cancer model of pain. *Pharmacol Biochem Behav*. **86**:458-67.

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8.12. Tomić MA, Vucković SM, Stepanović-Petrović RM, Ugresić ND, Prostran MS, Bosković B.(2010). Synergistic interactions between paracetamol and oxcarbazepine in somatic and visceral pain models in rodents. *Anesth Analg.***110**:1198-205.

8.13. Zhang Y, Du L, Pan H, Li L, Su X. (2011). Enhanced analgesic effects of propacetamol and tramadol combination in rats and mice. *Biol Pharm Bull*. **34**:349-353.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2015.04.22	6.1 (NO TEXT)	6.1 **Use lidocaine HCl 2% (20mg/ml) injectable solution.**
2015.04.22	6.1 (NO TEXT)	6.1 **Use bupivacaine HCl 0.50% (5mg/ml) injectable solution.**
2015.04.22	6.1 (NO TEXT)	6.1 Lidocaine-bupivacaine mixture: **Discard mixture after 3 months.**
2015.04.22	7. (NO TEXT)	7. Mouse / Hamster / Gerbil: **Use carprofen 50mg/ml injectable solution.** **To prepare a 5mg/ml dilution: add 1ml of carprofen 50mg/ml to 9 ml of sterile water for injection. Administer 0.1-0.2ml/100g of bodyweight. Discard dilution after 3 months.** **Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.**
2015.04.22	7. (NO TEXT)	7. Rat and Guinea Pig / Chichilla: **Use carprofen 50mg/ml injectable solution.** **To prepare a 5mg/ml dilution: add 1ml of carprofen 50mg/ml to 9 ml of sterile water for injection. Administer 0.1-0.2ml/100g of bodyweight. Discard dilution after 3 months.**
2016.02.01	7. (NO TEXT)	**Buprenorphine Slow Release (SR)** **Dose: 1mg/kg** **Route of administration: SC** **Frequency: once** **Note: Buprenorphine SR (0.6mg/ml) is a sustained release buprenorphine product that has been developed to provide 72 hours of analgesia and is specifically designed for use in rodents. See administration instructions in section 7.2.** **Controlled drug.**
2016.02.01	7.2 (NO TEXT)	**7.2. Administration instructions for buprenorphine slow release (SR):** **7.2.1. Avoid contact with the skin to prevent the development of injection site reactions:** **7.2.2. For use in mice: draw up the buprenorphine SR with a 25g or 27g needle attached to a 1cc low waste syringe (alternatively, use a 28g insulin syringe) and wipe needle off with an alcohol swab to remove any drug residue from the needle prior to administration.** **7.2.3.F or use in rats: draw up the buprenorphine SR with an 18g needle and change to a 23g needle prior to administration** **7.2.4. Administer subcutaneously on back of neck between the shoulder blades while the animal is anesthetized.** **7.2.5. Administer slowly and finish injecting before the needle is pulled out through skin.** **7.2.6. Pinch the injection site for approximately 10 seconds after removing the needle** **7.2.7. Do not combine the buprenorphine SR with any other drugs and do not attempt to dilute the formulation.**
2017.01.27	7.1 (NO TEXT)	**7.1. Administration of non-steroidal anti-inflammatory drugs (NSAIDs):** **7.1.1. NSAIDs include carprofen, ketoprofen and meloxicam.** **7.1.2. Ensure good water intake and monitor hydration status during the treatment period.** **7.1.3. Suspend water restriction prior to administration of NSAIDs.** **7.1.4. To minimize chances for adverse drug interactions, a washout period of 5-7 days is recommended before switching between NSAIDs.**
2017.01.27	4.5 and 4.6 Grimace Scale Image	4.5 and 4.6 Grimace Scale Image **replaced with NC3Rs poster image**
2018.10.15	7.2.7. Do not combine the buprenorphine SR with any other drugs and do not attempt to dilute the formulation.	7.2.7. Do not combine the buprenorphine SR with any other drugs **in the same syringe** and do not attempt to dilute the formulation.
2018.11.12	7. (NO TEXT)	7. General Analgesia: Tramadol removed as its use in mice and rats as an oral dosage alone is not recommended.
2018.11.12	7. Mouse / Hamster / Gerbil: Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.	7. Mouse / Hamster / Gerbil: ~~Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.~~
2018.11.12	7. (NO TEXT)	7. Rats, Guinea Pig / Chinchilla: **Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.**
2018.11.12	7. (NO TEXT)	7. Mouse / Hamster / Gerbil, Rats, Guinea Pig / Chinchilla: Acetaminophen **Used in conjunction with buprenorphine enhances pain relief.** **Provide acetaminophen-treated drinking water at least 24 hours in advance of painful procedure.** **Prepare solution by mixing Children's Tylenol suspension liquid to drinking water. Mix bottle thoroughly before use.** **Fresh solution should be prepared and added to clean bottles every 3-4 days for maximum efficacy.**