

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 invasiveness of the procedure (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.



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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 <ul style="list-style-type: none">▪ Closing of the eyelid (narrowing of orbital area)▪ A wrinkle may be visible around the eye			
Nose bulge <ul style="list-style-type: none">▪ Bulging on the bridge of the nose▪ Vertical wrinkles on the side of the nose			
Cheek bulge <ul style="list-style-type: none">▪ Bulging of the cheeks			
Ear position <ul style="list-style-type: none">▪ 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 <ul style="list-style-type: none">▪ 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 DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML, Matsunaga L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, Mogil JS. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(9): 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

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.



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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 <ul style="list-style-type: none">▪ Closing of the eyelid (narrowing of orbital area)▪ A wrinkle may be visible around the eye			
Nose/cheek flattening <ul style="list-style-type: none">▪ Flattening and elongation of the bridge of the nose▪ Flattening of the cheeks (potentially sunken look)			
Ear changes <ul style="list-style-type: none">▪ Ears curl inwards and are angled forward to form a 'pointed' shape▪ Space between the ears increases			
Whisker change <ul style="list-style-type: none">▪ 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

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, a combination of opioid, non-steroidal anti-inflammatory drug (NSAID), and infiltration of a local analgesic.
- 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	Route	Duration	Note
Lidocaine	< 2 mg/kg	SC, Infiltration of surgical wounds	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 must be prepared immediately before use and should not be stored. Diluted solution is as effective but induction of analgesia is slightly prolonged. *Dilution with sodium bicarbonate is not necessary if lidocaine is to be administered to an anesthetized animal.
Bupivacaine	< 2 mg/kg	SC, Infiltration of surgical wounds	3–4 hrs.	Use bupivacaine HCl 0.50% (5mg/ml) injectable solution. Same comment as for lidocaine.
* Lidocaine-bupivacaine mixture	< 2 mg/kg	SC, Infiltration of surgical wounds	30min. to 4 hrs.	Same comment as for lidocaine. Combining both drugs allows for rapid induction and prolonged effect. Use a 1:1 mixture of lidocaine HCl 2% (20mg/ml) injectable solution and bupivacaine HCl 0.50% (5mg/ml) injectable solution. Discard mixture after 3 months.
EMLA cream	Thick spread	Topical	30–60 min.	Shave or pluck the fur and apply a thick layer of cream ideally 10 minutes before the painful procedure. Apply only to intact skin.

*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. Controlled drug. 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. Store at room temperature. Discard dilution after 1 month.
*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

Mouse Neonates

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine	0.05-0.1 mg/kg	SC, IP	4–8 hrs.	Mild to moderate pain. Controlled drug.
Morphine	5-10 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. Controlled drug. See administration instructions in section 7.2.
*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. Store at room temperature. Discard dilution after 1 month.
*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

Rat Neonates

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine	0.01-0.05 mg/kg	SC, IP	4–8 hrs.	Mild to moderate pain. Controlled drug.
Morphine	5-10 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. Store at room temperature. Discard dilution after 1 month. 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.
- 7.1.5. Do not administer NSAIDs to neonatal rodents.

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, preferably while the animal is anesthetized.
- 7.2.5. Administer slowly and finish injecting before the needle is pulled out.
- 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

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SOP REVISION HISTORY

DATE	NEW VERSION
2015.04.22	6.1 Use lidocaine HCl 2% (20mg/ml) injectable solution.
2015.04.22	6.1 Use bupivacaine HCl 0.50% (5mg/ml) injectable solution.
2015.04.22	6.1 Lidocaine-bupivacaine mixture: Discard mixture after 3 months.
2015.04.22	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. 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	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. 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 and do not attempt to dilute the formulation.
2017.01.27	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 replaced with NC3Rs poster image
2018.10.15	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. 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.
2018.11.12	7. Rats, Guinea Pig / Chinchilla: Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.
2018.11.12	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.
2019.11.24	6.1 Lidocaine-bupivacaine mixture: Use a 1:1 mixture of lidocaine HCl 2% (20mg/ml) injectable solution and bupivacaine HCl 0.50% (5mg/ml) injectable solution.
2019.11.24	[Added analgesics doses for mouse and rat neonates]
2019.11.24	7.1.5. Do not administer NSAIDs to neonatal rodents.
2019.11.24	8.15. Simonek GD, Alarcio GG, Brignolo LL. Sterility and Stability of Diluted Carprofen in a Multidose Vial in the Laboratory Animal Setting. J Am Assoc Lab Anim Sci. 2017;56(3):296–298. 8.16. Perinatal Studies - Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. National Research Council (US) Committee on Guideline for the Use of Animals in Neuroscience and Behavioral Research. Washington (DC): National Academies Press (US); 2003. 7, Perinatal Studies. Available from: https://www.ncbi.nlm.nih.gov/books/NBK43316/
2020.01.06	7. Carprofen: Store at room temperature. Discard dilution after 3 months.
2020.03.23	4.1. Adapt the frequency of observation to the proto el invasiveness of the procedure (minimum once a day).
2020.03.23	6.1 (Added routes for local analgesics)
2020.03.23	6.1 EMLA cream: Apply only to intact skin.
2020.03.23	7. Rat: Carprofen Carprofen oral gel (from Clear H2O) can be used after an initial subcutaneous dose.
2020.03.23	7.2.4. Administer subcutaneously on back of neck between the shoulder blades, preferably while the animal is anesthetized.
2021.11.01	5.2. Use a combination of analgesics, which is often more effective than using a single agent. For example, a combination of buprenorphine opioid, carprofen non-steroidal anti-inflammatory drug (NSAID), and local infiltration of lidocaine/bupivacaine-a local analgesic.