

**1. PURPOSE**

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

**2. RESPONSIBILITY**

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Principal investigators (PI) and their staff, veterinarians, veterinary and animal care staff.

**3. GENERAL CONSIDERATIONS**

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- 3.1. A procedure which would be expected to be painful if it were done on human 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**

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- 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. Wild animals will usually hide any signs of pain. Common clinical signs indicative of pain or distress include:
  - 4.4.1. Avoidance
  - 4.4.2. Vocalization
  - 4.4.3. Eyebrow movements
  - 4.4.4. Escape and aggressiveness
  - 4.4.5. Spontaneous activities are reduced; the animal is isolated from the social group
  - 4.4.6. Apathy, anxiety, plaintive
  - 4.4.7. Altered gait
  - 4.4.8. Nibbling, licking, scratching, rubbing
  - 4.4.9. Eyes are semi-closed
  - 4.4.10. Head shaking (ear pain)
  - 4.4.11. 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. Use the Cynomolgus Macaque Grimace Scale (Paterson et al., 2023). Grimace scales are one means of assessing the occurrence of acute pain using action units such as facial expressions and posture.

## 5. ANALGESIA PLAN

- 5.1. Analgesia should be provided 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	30 min. 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 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

### Macaques

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine	0.005–0.03 mg/kg	IM (preferred) IV, SC	6-8 hrs (low dose) 8–12 hrs (high dose)	Controlled drug.

### Macaques

Analgesic	Dose	Route	Frequency	Note
Buprenorphine Slow Release (SR)	0.2 mg/kg	SC	48-72 hrs	Buprenorphine SR is a sustained release buprenorphine product that has been developed to provide up to 72 hours of analgesia in NHPs. See administration instructions in section 7.1.  Controlled drug.
Carprofen	2-4 mg/kg	SC, IM	12-24 hrs.	4mg/kg first dose, then 2mg/kg.
Fentanyl	0.5-0.15 µg/kg (bolus)  7-20 µg/kg/hr (constant rate infusion)	IM  IV	Bolus  Constant rate infusion	For moderate to severe pain. Controlled drug
Ketoprofen	1–2 mg/kg	SC, IM, IV	12–24 hrs.	2mg/kg first day, then 1mg/kg.
*Meloxicam	0.1-0.2 mg/kg	SC, PO	24 hrs.	0.2mg/kg first day, then 0.1mg/kg.
Meloxicam Slow Release (SR)	0.6 mg/kg	SC	48-72 hrs	Meloxicam SR (2mg/ml) is a sustained release meloxicam product that has been developed to provide up to 72 hours of analgesia in NHPs. See administration instructions in section 7.2.
Morphine-Lidocaine-Ketamine Combination	2 ml/kg/hr	IV	Constant rate infusion	To a 500ml bag of fluids, add morphine 60mg, lidocaine 750mg and ketamine 150mg. Controlled drugs.

\*most commonly used

### Marmosets

Analgesic	Dose	Route	Frequency	Note
Carprofen	2-4 mg/kg	SC, PO	12-24 hrs.	4mg/kg first dose, then 2mg/kg.
Buprenorphine	0.005 – 0.01 mg/kg	IM, SC	6-8 hrs (low dose)  8–12 hrs (high dose)	Controlled drug.
Buprenorphine Slow Release (SR)	0.2 mg/kg	SC	48-72 hrs	Buprenorphine SR is a sustained release buprenorphine product that has been developed to provide up to 72 hours of analgesia in NHPs. See administration instructions in section 7.1.  Controlled drug.
Meloxicam	0.1-0.2 mg/kg	SC, PO	24 hrs.	0.2mg/kg first day, then 0.1mg/kg.

- 7.1. Administration instructions for buprenorphine slow release (SR):
  - 7.1.1. Avoid contact with the skin to prevent the development of injection site reactions.
  - 7.1.2. Use a 23 G needle to draw up and administer the buprenorphine SR.
  - 7.1.3. Administer slowly and finish injecting before the needle is pulled out.
  - 7.1.4. Pinch the injection site for approximately 10 seconds after removing the needle.
  - 7.1.5. Do not combine the buprenorphine SR with any other drugs in the same syringe and do not attempt to dilute the formulation.
- 7.2. Administration of non-steroidal anti-inflammatory drugs (NSAIDs):
  - 7.2.1. NSAIDs include carprofen, ketoprofen and meloxicam.
  - 7.2.2. Ensure good water intake and monitor hydration status during the treatment period.
  - 7.2.3. Suspend water restriction prior to administration of NSAIDs.
  - 7.2.4. To minimize chances for adverse drug interactions, a washout period of 5-7 days is recommended before switching between NSAIDs.

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## 8. REFERENCES

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- 8.2. Mackiewicz AL, Salyards GW, Knych HK, Hill AE, Christe KL. Pharmacokinetics of a Long-lasting, Highly Concentrated Buprenorphine Solution after Subcutaneous Administration in Rhesus Macaques (*Macaca mulatta*). *J Am Assoc Lab Anim Sci*. 2019;58(4):501–509. [doi:10.30802/AALAS-JAALAS-18-000115](https://doi.org/10.30802/AALAS-JAALAS-18-000115).
- 8.3. DiVincenti L Jr. Analgesic use in nonhuman primates undergoing neurosurgical procedures. *J Am Assoc Lab Anim Sci*. 2013 Jan;52(1):10-6. PMID: 23562027; PMCID: [PMC3548195](https://pubmed.ncbi.nlm.nih.gov/23562027/).
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- 8.5. Sabrina R. Bourgeois, Maribel Vazquez & Kathleen Brasky (2007) Combination Therapy Reduces Self-injurious Behavior in a Chimpanzee (*Pan Troglodytes Troglodytes*): A Case Report, *Journal of Applied Animal Welfare Science*, 10:2, 123-140, [DOI: 10.1080/10888700701313454](https://doi.org/10.1080/10888700701313454).
- 8.6. Fabian, N. J., Moody, D. E., Averin, O., Fang, W. B., Jamiel, M., Fox, J. G., Burns, M. A., & Haupt, J. L. (2021). Pharmacokinetics of Single-Dose Intramuscular and Subcutaneous Injections of Buprenorphine in Common Marmosets (*Callithrix jacchus*). *Journal of the American Association for Laboratory Animal Science : JAALAS*, 60(5), 568–575. <https://doi.org/10.30802/AALAS-JAALAS-20-000151>
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- 8.8. Carpenter J.W., Harms C. *Carpenter's Exotic Animal Formulary*, 6th Edition, September 2022.

## 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.
2016.02.01	7. Buprenorphine route of administration: IM, SC
2016.02.01	7. Meloxicam route of administration: SC, PO
2016.02.01	7. Buprenorphine dose: 0.005– <del>0.01</del> 0.02 mg/kg
2016.02.01	7. General analgesia Macaque <b>Buprenorphine Slow Release (SR)</b> Dose: 0.2 mg/kg Route of administration: SC Frequency: 72-120 hrs Note: Buprenorphine SR (3mg/ml) is a sustained release buprenorphine product that has been developed to provide up to 120 hours of analgesia in NHPs. See administration instructions in section 7.1. Controlled drug.
2016.02.01	7.1. Administration instructions for buprenorphine slow release (SR): 7.1.1. Avoid contact with the skin to prevent the development of injection site reactions. 7.1.2. For use in NHPs: draw up the buprenorphine SR with an 18G needle and change to a 23g needle prior to administration. 7.1.3. Administer slowly and finish injecting before the needle is pulled out through skin. 7.1.4. Pinch the injection site for approximately 10 seconds after removing the needle. 7.1.5. Do not combine in the same syringe the buprenorphine SR with any other drugs and do not attempt to dilute the formulation.
2016.09.02	7. Carprofen, ketoprofen and meloxicam: Ensure good water intake and monitor hydration status. Suspend water restriction prior to administration.
2016.09.02	Buprenorphine route of administration: IM (preferred), IV, SC
2016.09.02	5.2 For example, administer a combination of buprenorphine, <del>ketoprofen</del> carprofen, and local infiltration of <del>lidocaine</del> a local analgesic.
2017.01.27	7.2. Administration of non-steroidal anti-inflammatory drugs (NSAIDs): 7.2.1. NSAIDs include carprofen, ketoprofen and meloxicam. 7.2.2. Ensure good water intake and monitor hydration status during the treatment period. 7.2.3. Suspend water restriction prior to administration of NSAIDs. 7.2.4. To minimize chances for adverse drug interactions, a washout period of 5-7 days is recommended before switching between NSAIDs.
2018.10.12	7.1.5. Do not combine in the same syringe the buprenorphine SR with any other drugs in the same syringe and do not attempt to dilute the formulation.
2020.04.20	4.1. Adapt the frequency of observation to the <del>protocol</del> invasiveness of the procedure (minimum once a day).
2020.04.20	4.4. Wild animals will usually hide any signs of pain. Common clinical signs indicative of pain or distress include: 4.4.1. Avoidance 4.4.2. Vocalization 4.4.3. Eyebrow movements 4.4.4. Escape and aggressiveness 4.4.5. Spontaneous activities are reduced; the animal is isolated from the social group 4.4.6. Apathy, anxiety, plaintive 4.4.7. Altered gait 4.4.8. Nibbling, licking, scratching, rubbing 4.4.9. Eyes are semi-closed 4.4.10. Head shaking (ear pain) 4.4.11. Reduced appetite and subsequent weight loss
2020.04.20	6.1 (Added routes for local analgesics)
2020.04.20	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.
2020.04.20	6.1 EMLA cream: Apply only to intact skin.
2020.04.20	7. Buprenorphine Slow Release (SR) frequency: <del>72-120</del> 48-72 hours
2020.04.20	8.2. Mackiewicz AL, Salyards GW, Knych HK, Hill AE, Christe KL. Pharmacokinetics of a Long-lasting, Highly Concentrated Buprenorphine Solution after Subcutaneous Administration in Rhesus Macaques (Macaca mulatta). J Am Assoc Lab Anim Sci. 2019;58(4):501–509. doi:10.30802/AALAS-JAALAS-18-000115
2021.11.01	5.2. <del>Try to</del> Use a combination of analgesics, which is often more effective than using a single agent. For example, a combination of <del>buprenorphine</del> opioid, <del>carprofen</del> non-steroidal anti-inflammatory drug (NSAID), and local infiltration of <del>lidocaine/bupivacaine</del> a local analgesic.
2021.11.01	Morphine. Dose: 1-2 mg/kg, Route: SC or IM, Frequency: 4 hrs, Notes: For moderate to severe pain. Controlled drug.
2021.11.01	Meloxicam Slow Release (SR). Dose: 0.6 mg/kg, Route: SC, Frequency: 48-72 hrs, Notes: Meloxicam SR (2mg/ml) is a sustained release meloxicam product that has been developed to provide up to 72 hours of analgesia in NHPs. See administration instructions in section 7.1.

2021.11.01	Fentanyl. Dose: 0.5-0.15 µg/kg (bolus), 7-10 µg/kg/hr (constant rate infusion), Route: IM Bolus, IV Constant rate infusion, Notes: For moderate to severe pain. Controlled drug.
2021.11.01	Gabapentin. Dose: 10-50 mg/kg, Route: PO, Frequency: 8-12 hrs, Notes: Anticonvulsant with some analgesic properties. Should be used for multimodal analgesia and not as a single analgesic agent.
2021.11.01	[Added section specifically for marmosets]
2021.11.01	7.1.2. Use a 23g needle to draw up and administer the buprenorphine SR with an 18g needle and change to a 23g needle prior to administration.
2021.11.01	8.3. DiVincenti L Jr. Analgesic use in nonhuman primates undergoing neurosurgical procedures. J Am Assoc Lab Anim Sci. 2013 Jan;52(1):10-6. PMID: 23562027; PMCID: PMC3548195. 8.4. Radulovic LL, Türck D, von Hodenberg A, Vollmer KO, McNally WP, DeHart PD, Hanson BJ, Bockbrader HN, Chang T. Disposition of gabapentin (neurontin) in mice, rats, dogs, and monkeys. Drug Metab Dispos. 1995 Apr;23(4):441-8. PMID: 7600909. 8.5. Sabrina R. Bourgeois, Maribel Vazquez & Kathleen Brasky (2007) Combination Therapy Reduces Self-injurious Behavior in a Chimpanzee (Pan Troglodytes Troglodytes): A Case Report, Journal of Applied Animal Welfare Science, 10:2, 123-140, DOI: 10.1080/10888700701313454.
2022.03.08	7. Acetaminophen. Dose: 15 mg/kg, Route: PO, Frequency: 4-6 hrs., Notes: For mild pain.
2022.03.08	7. Gabapentin. Dose: 10-50 mg/kg, Route: PO, Frequency: 8-12 hrs, Notes: Anticonvulsant with some analgesic properties. Should be used for multimodal analgesia and not as a single analgesic agent.
2022.03.08	7. Ketamine. Dose: 0.1-1.0 mg/kg, After initial bolus, constant rate infusion of 0.1-0.3 mg/kg/hr, Route: SC, IM, IV, Notes: Controlled drug.
2023.07.28	7. General analgesia (Marmoset) Buprenorphine Slow Release (SR) Dose: <del>0.03-0.1</del> 0.2 mg/kg
2023.07.28	8.6. Fabian, N. J., Moody, D. E., Averin, O., Fang, W. B., Jamiel, M., Fox, J. G., Burns, M. A., & Haupt, J. L. (2021). Pharmacokinetics of Single-Dose Intramuscular and Subcutaneous Injections of Buprenorphine in Common Marmosets (Callithrix jacchus). Journal of the American Association for Laboratory Animal Science : JAALAS, 60(5), 568–575. <a href="https://doi.org/10.30802/AALAS-JAALAS-20-000151">https://doi.org/10.30802/AALAS-JAALAS-20-000151</a>
2023.08.14	2. Responsibility Principal investigators (PI) and their staff, veterinarians, veterinary and animal care staff, and any personnel who will monitor animals undergoing potentially painful procedures.
2023.08.14	4.5. Use the Cynomolgus Macaque Grimace Scale (Paterson et al., 2023). Grimace scales are one means of assessing the occurrence of acute pain using action units such as facial expressions and posture.
2023.08.14	5.1. If possible, provide Analgesia should be provided before the painful stimulus, as it is more effective in preventing pain (e.g. give analgesic before surgery).
2023.08.14	7. General analgesia (Macaques) Fentanyl. Dose: 0.5-0.15 µg/kg (bolus), 7-10 20 µg/kg/hr (constant rate infusion)
2023.08.14	7. General analgesia (Macaques) Buprenorphine. Dose: 0.005- <del>0.02</del> 0.03 mg/kg
2023.08.14	7. General analgesia (Macaques) Fentanyl Patch. Dose: 25µg patch (3-7kg BW), Frequency: Every 3 days, starting 24h prior to surgery, Note: Protect patch under a jacket. For moderate to severe pain. Controlled drug
2023.08.14	7. General analgesia (Macaques) <del>Morphine. Dose: 1-2 mg/kg, Route: SC, IM, IV, Frequency: 4 hrs, Notes: For moderate to severe pain. Controlled drug.</del>
2023.08.14	8.7. Paterson, E.A., O'Malley, C.I., Moody, C. et al. Development and validation of a cynomolgus macaque grimace scale for acute pain assessment. Sci Rep 13, 3209 (2023). <a href="https://doi.org/10.1038/s41598-023-30380-x">https://doi.org/10.1038/s41598-023-30380-x</a>
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