

1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe methods of assessing pain in fish, aquatic amphibians and reptiles, 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.
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.
- 4.4. Fish, amphibians, and reptiles do not exhibit obvious clinical signs of pain. Because fish and amphibians can experience pain as mammals do, the assumption is made by extrapolation from human observation.

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. Try to use a combination of analgesics, which is often more effective than using a single agent.
For example, combination of opioid, 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.

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 hr.	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.
Lidocaine or benzocaine (Orajel) gel	-	Topical	30–60 min.	For fish and amphibians only. Apply a thin layer over the affected area

*most commonly used

7. GENERAL ANALGESIA

Fish

Analgesic	Dose	Route	Frequency	Note
Lidocaine	2-5 mg/L	Immersion	Immersion	
*MS222	25-300 mg/L	Immersion	Immersion	
Morphine	10 mg/ L	Immersion	Immersion	Controlled drug.

Amphibians

Analgesic	Dose	Route	Frequency	Note
Buprenorphine	75 mg/kg	SC	> 4 hr.	Controlled drug.

Reptiles

Analgesic	Dose	Route	Frequency	Note
*Buprenorphine	0.01 mg/kg	IM	q12/24 hr.	Controlled drug.
*Carprofen	2-4 mg/kg	PO, SC, IM	q24 hr.	Followed by 1-2 mg/kg q24-72 hr.
Ketoprofen	2 mg/kg	SC, IM	q24-48 hr.	
Meloxicam	0.1-0.2 mg/kg	PO, SC, IM	q24 hr.	

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

8. SAFETY PRACTICES

- 8.1. MS-222:
 - 8.1.1. Wear protective clothing, gloves, and eye protection when handling the MS-222 powder.
 - 8.1.2. Wear gloves to handle animals exposed to MS-222
 - 8.1.3. Making MS-222 solutions:
 - 8.1.3.1. Contact Environmental Health and Safety Department for safe handling, use, and storage procedures.
 - 8.1.4. Disposal of MS-222 waste:
 - 8.1.4.1. Contact the Waste Management department for disposal procedures.

9. REFERENCES

- 9.1. Chatigny F, Kamunde C, Creighton CM, Stevens ED. Uses and Doses of Local Anesthetics in Fish, Amphibians, and Reptiles. *J Am Assoc Lab Anim Sci.* 2017;56(3):244–253.
- 9.2. Hannon D. Non-Steroidal Anti-inflammatory Drugs (NSAIDs) in Reptiles and Amphibians: A Review. ExoticsCon 2015 Main Conference Proceedings.
<https://pdfs.semanticscholar.org/97e4/c202f32a60de4e61d18992fba107bf8ddf21.pdf>
- 9.3. Canadian Council on Animal Care. [CCAC guidelines: Zebrafish and other small, warm-water laboratory fish](#) (2020).
- 9.4. Chatigny F, Creighton CM, Stevens ED. Updated Review of Fish Analgesia. *J Am Assoc Lab Anim Sci.* 2018 Jan 1;57(1):5-12. PMID: 29402345; PMCID: [PMC5875091](#).
- 9.5. Canadian Council on Animal Care. [CCAC guidelines: Zebrafish and other small, warm-water laboratory fish](#) (2020).

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.
2016.09.06	7. Carprofen, ketoprofen and meloxicam: Ensure good water intake and monitor hydration status. Suspend water restriction prior to administration.
2016.09.06	5.2 For example, administer a combination of buprenorphine, ketoprofen carprofen, and local infiltration of lidocaine a local analgesic.
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.
2020.04.20	4.1. Adapt the frequency of observation to the protocol invasiveness of the procedure (minimum once a day).
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	8.1. Chatigny F, Kamunde C, Creighton CM, Stevens ED. Uses and Doses of Local Anesthetics in Fish, Amphibians, and Reptiles. J Am Assoc Lab Anim Sci. 2017;56(3):244–253. 8.2. Hannon D. Non-Steroidal Anti-inflammatory Drugs (NSAIDs) in Reptiles and Amphibians: A Review. ExoticsCon 2015 Main Conference Proceedings. https://pdfs.semanticscholar.org/97e4/c202f32a60de4e61d18992fba107bf8ddf21.pdf
2020.05.13	7. Buprenorphine frequency of monitoring: immersion q12-24 hr.
2020.05.13	7. Carprofen frequency of monitoring: q24 hr.
2020.12.07	8. SAFETY PRACTICES 8.1. MS-222: 8.1.1. Wear protective clothing, gloves and goggles when handling the MS-222 powder. 8.1.2. Wear gloves to handle animals exposed to MS-222 8.1.3. Making MS-222 solutions: 8.1.3.1. Work inside a fume hood to prepare a concentrated stock solution by mixing an appropriate amount of MS-222 powder in a small volume of water. 8.1.3.2. Dilute the stock solution further as required. 8.1.3.3. Wear gloves and use a utensil to stir until all powder is dissolved. 8.1.4. Disposal of MS-222 waste: 8.1.4.1. MS-222 should be collected and disposed of as chemical waste. Contact the Waste Management department for details. 8.1.4.2. Do not discard MS-222 directly into sinks, drains, surface water, storm water conveyances or catch basins.
2021.11.03	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.
2021.11.03	7. General Analgesia, Fish. Lidocaine. Dose: 300-325 mg/L, Route: Immersion, Frequency: Immersion
2021.11.03	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.
2021.11.03	9.3. Canadian Council on Animal Care. CCAC guidelines: Zebrafish and other small, warm-water laboratory fish (2020). 9.4. Chatigny F, Creighton CM, Stevens ED. Updated Review of Fish Analgesia. J Am Assoc Lab Anim Sci. 2018 Jan 1;57(1):5-12. PMID: 29402345; PMCID: PMC5875091
2021.11.11	8.1.1. Wear protective clothing, gloves, and goggles eye protection when handling the MS-222 powder. 8.1.2. Wear gloves to handle animals exposed to MS-222 8.1.3. Making MS-222 solutions: 8.1.3.1. Work inside a fume hood to prepare a concentrated stock solution by mixing an appropriate amount of MS-222 powder in a small volume of water. 8.1.3.2. Dilute the stock solution further as required. 8.1.3.3. Wear gloves and use a utensil to stir until all powder is dissolved. 8.1.3.1. Contact Environment Health and Safety Department for safe handling, use, and storage procedures. 8.1.4. Disposal of MS-222 waste: 8.1.4.1. MS-222 should be collected and disposed of as chemical waste. Contact the Waste Management department for details. 8.1.4.2. Do not discard MS-222 directly into sinks, drains, surface water, storm water conveyances or catch basins. 8.1.4.1. Contact the Waste Management department for disposal procedures.
2021.11.11	9.5. Canadian Council on Animal Care. CCAC guidelines: Zebrafish and other small, warm-water laboratory fish (2020).
2022.01.13	The intent of this Standard Operating Procedure (SOP) is to describe methods of assessing pain in fish, aquatic amphibians, and reptiles, and mitigating pain by administration of analgesic medications.