



1. PURPOSE

This Standard Operating Procedure (SOP) intends to describe methods of assessing pain in fish, aquatic amphibians, and reptiles and mitigating pain by administering analgesic medications.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. GENERAL CONSIDERATIONS

- 3.1. A procedure expected to be painful in humans is considered painful in animals.
 - 3.2. When there is a question of whether a procedure is painful, the animal should benefit from 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 BGU ethical committee.
 - 3.5. Refer to SOP102b for additional Analgesia options in *Xenopus laevis*.
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4. PAIN RECOGNITION AND ASSESSMENT

- 4.1. Adapt the observation frequency to the procedure's invasiveness (minimum once a day).
- 4.2. Start by observing the animal from a distance, so the observer's presence does not alter the animal's behavior. Then proceed to keep the animal more closely.
- 4.3. Look for any changes in the behavior. Report animals that appear to be in pain to the vets.
- 4.4. Although fish, amphibians, and reptiles **do not** exhibit obvious clinical signs of pain, they can experience pain like mammals. Thus, the assumption is made by extrapolation from human observation.

Note: *The most reliable signs of pain and distress are changes in behavior.*

5. ANALGESIA PLAN

- 5.1. Specify the analgesia plan in your animal protocol.
- 5.2. Provide analgesia before the painful stimulus whenever possible, as it is more effective in preventing pain (e.g., give analgesic before surgery).

5.3. Use a combination of analgesics, often more effective than a single agent—for example, non-steroidal anti-inflammatory drugs (NSAID) and infiltration of a local analgesic.

6. LOCAL ANESTHESIA

6.1. Infiltrate or apply local analgesics to areas where a painful stimulus is induced. Repeat the application of local agents at specified intervals to maintain analgesia. In some cases, a sedative is recommended when using regional 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. A diluted solution is as effective, but induction of analgesia is slightly prolonged. *Dilution with sodium bicarbonate is unnecessary if lidocaine is administered to an anesthetized animal.
	EMLA cream	Topical	30–60 min.	Thick spread. Apply only to intact skin. Shave or pluck the fur and apply a thick layer of cream, ideally 10 minutes before the painful procedure.
	Lidocaine 2% gel or cream			
	Eye drops	Ocular	30-60min	
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.

7. SYSTEMIC ANALGESIA

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

7.1.1. NSAIDs include carprofen, ketoprofen, and meloxicam.

7.1.2. A washout period of 5-7 days is recommended to minimize chances for adverse drug interactions before switching between NSAIDs.

7.1.3. Ensure good water intake and monitor hydration status during treatment.

Fish

Analgesic	Dose	Route	Frequency	Note
Lidocaine	2-5 mg/L	Immersion	Immersion	
*MS222	25-300 mg/L	Immersion	Immersion	

Amphibians

Analgesic	Dose	Route	Frequency	Note
Lidocaine	2-5 mg/L	Local injection	Just before surgery	Anesthetized animal
Flunixin	25 mg/kg	Intracelomic	Once	

Reptiles

Analgesic	Dose	Route	Frequency	Note
*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.	

8. SAFETY PRACTICES

8.1. MS-222:

- 8.1.1. Wear protective clothing, gloves, and eye protection, or work in a chemical fume hood when handling the MS-222 powder.
- 8.1.2. Wear gloves to handle animals exposed to MS-222.

SOP 102 FISH, AMPHIBIAN, AND REPTILE ANALGESIA

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Approved by the BGU Animal Policy and Welfare Oversight Committee