Guidelines on Amphibian Anesthesia Analgesia and Surgery

Last Updated 15 March 2016

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1. Purpose

1. This document has been designed by the ULAM veterinary staff as a guideline for sedation, anesthesia, and analgesia of amphibians, specifically Xenopus sp. This is not intended to be an inclusive tutorial on all possible drug combinations that can be used in amphibians. The following guidelines are also general recommendations and consequently do not include reference to specific research associated concerns. If you have questions or comments about this document, please contact the ULAM veterinary staff at ulam-vets@umich.edu or 734-936-1696. The ULAM training core (ULAM-trainingcore@umich.edu or 734-763-8039) can be contacted to provide training in techniques at no charge.

2. All surgical procedures, anesthetics, analgesics, antibiotics or other medications used on animals must be approved by the IACUC, described in the animal use protocol and performed by personnel listed on the protocol and appropriately trained for the surgical procedure.

3. Any techniques or drug protocols deviating from this document must be justified and approved in the IACUC protocol prior to application.

4. More information on appropriate injection techniques and volumes can be found in the Guidelines on Administration of Substances to Laboratory Animals.

5. More specific information regarding monitoring procedures can be found in the ULAM Anesthesia and Sedation Monitoring Guidelines.

6. More specific information regarding anesthetic, sedation and analgesic drug classes can be found in the ULAM Anesthetic and Analgesic Drug Description.

7. For any concerns regarding animal health after work hours or on holidays/weekends, please contact DPS (3-1131) who will contact the on-call veterinarian.

2. Responsibility

1. Principal Investigator: Responsible to ensure appropriate anesthesia, monitoring, and analgesia is provided for all animals undergoing surgical or sedation procedures.

3. Definitions

1. Anesthesia: Temporarily induces loss of sensation with or without loss of consciousness.


3. A/A: Anesthesia and analgesia.

4. Sedation: A mild degree of central depression in which the patient is awake but calm.

5. Immersion: A method of delivering drugs via direct contact with the skin in a bath.

4. Procedures

1. Special Considerations in Amphibian Anesthesia

1. Appropriate anesthesia: Frogs have several adaptive features which make anesthesia challenging including the ability to exchange CO2 cutaneously, the ability to hold their breath for long periods of time, and unique tracheal anatomy making intubation and gas anesthesia generally unfeasible.

2. Specialized amphibian respiratory systems and highly absorbent integumentary systems allow the application of many anesthetic agents to the skin rather than via a parenteral route.
   1. Terrestrial amphibians respire through both lungs and skin.
   2. Aquatic amphibians respire through the skin.
   3. Urodeles (e.g. salamanders and newts) respire through both skin and gills.
3. Vascular access
   1. Blood vessels are not readily accessible in the frog. However, access to the cardiovascular system can be gained via injection into the dorsal lymph sacs located on either side of the urostyle. Under strong illumination, these can be seen pulsating through the skin. Please contact the ULAM Training Core for more information and to receive training on these techniques.

4. Fasting Periods: There is little information available on species specific fasting periods for amphibians. In general, short fasting periods are recommended (x < 24 hours) but please consult with ULAM veterinary staff for more specific recommendations in consideration of research aims and species of interest.

5. For a helpful tutorial on amphibian anesthesia, please refer to AAZA (American Association of Zoos and Aquariums) videos on amphibian medicine linked in the references (>).

2. Monitoring Amphibian Anesthetic Depth
   1. The following signs are listed in the order in which they occur during onset of anesthesia:
      1. Erythema (reddening) of skin of caudal abdomen
      2. Loss of righting reflex (inability to remain upright)
      3. Cessation of abdominal respiratory movements
      4. Cessation of spontaneous movement
      5. Loss of corneal reflex
      6. Loss of throat movements
      7. Cessation of movement in response to stimulation
   2. Cessation of spontaneous movement occurs earlier with MS-222 than other anesthetic agents.
   3. At the surgical plane of anesthesia, respiratory movements are greatly depressed to non-existent and there is no response to painful stimuli. However, cutaneous respiration is sufficient to maintain appropriate blood oxygen levels for most short procedures. Cardiac pulsation becomes more obvious as the muscles relax. Deeper anesthesia is associated with reduction in heart rate and then reduction in strength of the contraction. At deep planes of anesthesia which may indicate an anesthetic overdose, cardiac pulsation may be in-apparent.
   4. Advanced monitoring:
      1. Doppler: Small doppler probes may be used to evaluate heart rate in amphibians while under anesthesia. An 8.0 mHz unit with a 9-mm contact surface diameter placed directly over the heart on the ventrum has been successfully used in amphibians as small as 2 grams.
      2. Pulse oximetry: Small pulse oximetry clips may be directly attached to amphibian limbs to evaluate hemoglobin oxygen saturation. (Wright and Whitaker, 2001)

3. Preferred Anesthetic and Analgesic Agents
   1. General Anesthesia
      1. MS-222 (Tricaine Methanesulfonate) Recommended
      1. Use
         1. Pharmaceutical-grade MS-222 is required.
         2. Two pharmaceutical-grade MS-222 preparations are currently available:
            1. FINQUEL MS-222 - 99.5% Pure Tricaine Methanesulfonate
            2. Western Chemical's TRICAIN-E-S (MS-222, TMS, tricaine methanesulfonate) is an FDA approved amphibians anesthetic (FDA ANADA 200-226)
         2. MS-222 is a chemical powder that must be dissolved in water (fresh or salt) prior to usage. MS-222 is acidic in solution and must be buffered to a physiologic pH (7.0-7.4) prior to usage. Drug onset will be 10-20 minutes after immersion. Ensure water is properly aerated to prevent hypoxemia and observe animal at all times once in immersion bath as overdosing can readily occur. Solution should be rinsed away with fresh tank water when surgical anesthesia has been achieved.
         3. Induction times for amphibians vary, but generally a surgical plane of anesthesia will occur within 30 minutes of immersion into MS-222.
         4. If the animal begins to recover prior to completion of the procedure, there are several options:
            1. Apply a paper towel or gauze soaked in the original MS-222 solution directly to skin with care to avoid the surgical site.
            2. Drip the MS-222 solution directly onto the skin with care to avoid the surgical site.
            3. If there is no open incision, place the animal back into 50% of the original concentration of MS-222.
      2. Storage and Disposal
         1. MS-222 powder is stable for up to 5 years stored in the original sealed container in a dry location at temperatures x < 25°C.
         2. Stock Solutions
            1. Vendor recommendations concerning the stock solution stability and storage vary. It is recommended that all MS-222 stock solutions are utilized the same day as preparation. However, due to evidence from industry practices, stock solutions of MS-222 may be kept for 30 days and must be stored refrigerated in a dark or opaque bottle. Stock solutions of MS-222 must not be utilized for amphibian anesthesia if prepared more than 30 days prior or stored inaccurately in that interim. All MS-222 powder and stock solution containers must be appropriately stored, labeled (concentration and preparation or expiration date), and used prior to expiration date. (Alpharma, 2001 and Pharmaq, 2010)
            3. MS-222 solutions cannot be poured down the drain or introduced into the general water supply. Contact the University of Michigan's Occupational Safety and Environmental Health division (734 647-1143) for appropriate disposal methods.
      3. Safety Considerations
1. Preparation of the Surgical Area

4. Isoflurane
   1. Vaporized isoflurane (via induction chamber or bubbling through water) is considered effective but very short acting in amphibians and not appropriate for surgery.
   2. Topical isoflurane application has been shown to be effective in amphibians.
      1. 0.025 - 0.035 mL/g of 3cc/L of sterile lubricant/isoflurane mixture applied topically to the dorsum (Stevens, 2011)
         1. Mix with sterile lubricant jelly like KY-Jelly to a concentration of 3 cc/L by combining 3 cc liquid isoflurane, 1.5 cc water, and 3.5 cc KY-Jelly in a 10 cc conical tube mixed well
         2. Place the animal in a sealed container during induction and remove the anesthetic solution with a saline soaked gauze once an appropriate plane of anesthesia is reached (loss of withdrawal and righting reflex).
      3. Anesthetic duration varies from 45 - 80 minutes depending on concentration and individual species.
      4. Anesthetic induction and preparation of the isoflurane gel should occur within a fume hood or biosafety cabinet to prevent human exposure.

5. Benzocaine
   1. Benzocaine is the parent compound of MS-222 which is less water soluble and less acidic. It must be dissolved in acetone or ethanol.
   2. 0.025 % Benzocaine immersion (2.5 mL 10% benzocaine stock solution to 1 L of system water) for 10 - 15 minutes for anesthetic induction. Surgical anesthesia times vary from 15-30 minutes. (Stevens, 2011)

2. Sedation
   1. Benzocaine Gel
      1. Several commercially available preparations of benzocaine have been utilized for Xenopus sedation including:
         1. 7.5% Benzocaine Gel (Orajel® Regular)
         2. 20% Benzocaine Gel (Orajel® Maximum Strength or Anbesol®): Apply 0.5 - 1 cm length strip to the ventrum for anesthetic induction.
   2. Eugenol (Clove Oil)
      1. Use 300-350 mg/L immersion for 15 minutes for minimally invasive surgical procedures or for restraint.
      2. Eugenol has a widely variable anesthetic duration with a narrow margin of safety. Animals anesthetize with this compound often experience prolonged recoveries compared to MS-222. Direct application or use in high concentrations have been associated with dermal necrosis.
      3. Eugenol is not recommended for oocyte harvesting.
   3. Analgesic Agents
      1. There are currently no pharmacokinetically based recommendations regarding efficacious drug dosing of analgesics than can be safely administered to Xenopus. Limited lethality data suggest narrow safety indices for semi-terrestrial species. Analgesic use in fully aquatic species has the risk of drowning due to over sedation. Analgesic drugs and doses should be chosen and used very carefully. The following table is provided for guidance but ULAM veterinary consultation prior to usage is recommended.

4. Special Considerations in Amphibian Surgery

1. Surgical Site Preparation:
   1. Minimum Requirements: Rinse gross debris from the surgical site using sterile saline or another sterile isotonic fluid and throughout the surgical procedure keep the skin moist.
   2. Skin disinfection and draping: This remains controversial for many amphibian species and is not required. Do not use chlorohexadine or scrubs containing soaps or detergents as this may damage the skin. If skin disinfection is pursued, a 10% providone iodine solution is recommended. (Elsner et al., 2000) If a surgical drape is utilized, moisten the surgical drape prior to application with sterile saline to prevent skin over-drying.
   3. Place the animal in a sealed container during induction and remove the anesthetic solution with a saline soaked gauze once an appropriate plane of anesthesia is reached (loss of withdrawal and righting reflex).
   4. Anesthetic duration varies from 45 - 80 minutes depending on concentration and individual species.
   5. Anesthetic induction and preparation of the isoflurane gel should occur within a fume hood or biosafety cabinet to prevent human exposure.

5. Surgical Documentation
   1. Attached to this document is an Amphibian Anesthetic, Surgical and Post-Operative Monitoring Record. This is a template but at a minimum laboratory surgical records must include the same necessary information: animal ID, PI, protocol number, surgeon, procedure, number of prior procedures and date of last surgery (for oocyte harvesting procedures), any analgesic or anesthetic drugs used (including dosage, route, and frequency of administration), and a pre-surgical evaluation.

6. Preparation of the Surgical Area

1. According to the Guide for the Care and Use of Laboratory Animals: Eighth Edition, “For most survival surgery performed on rodents and other small species...the space should be dedicated to surgery and related activities when used for this purpose, and managed to minimize contamination from other activities conducted in the room at other times.” (pg. 144)
   1. The surgical area should be a room or a portion of a room that is easily sanitized and not used for any other purpose during the time of surgery.
2. Clean and disinfect the surface upon which the surgery will be performed with an approved environmental disinfectant before beginning the surgical procedure.

7. Preparation of Surgical Supplies

1. Surgical Instruments
   1. Use prepackaged aseptic surgical supplies whenever possible.
   2. Initial steam sterilization (autoclaving), plasma vapor sterilization, or ethylene oxide sterilization (for heat or pressure sensitive items) is required for all surgical instruments and items to be implanted.

2. Suture Materials and Incision Closure
   1. Monofilament suture like nylon or PDS is recommended to reduce inflammatory reaction in Xenopus skin.
   2. Close any laparotomy site in two layers (muscle and skin) to prevent incisional dehiscence.

8. Surgeon Preparation

1. Wash hands thoroughly with a disinfesting soap such as chlorhexidine or iodine based surgical scrubs or 3M Avaguard® hand antiseptic.
2. The surgeon must wear a mask, sterile or clean gloves, and a clean scrub top, clean disposable PPE gown, or clean lab jacket during the surgical procedure.
   1. Clean gloves include unused gloves stored in a sealable bag or container to minimize dust and debris contamination.
   2. Only non-powdered gloves should be used to manipulate amphibians to prevent skin damage.
   3. Avoid latex gloves with amphibians as adverse reactions have been recorded with their use. (Sobotka and Rahwan 1994; Gutleb et al., 2001)

9. Performing Multiple Surgeries in Series

1. Investigators should begin with at least 2 sets of sterile instruments.
   1. Between animals, clean the instruments followed by disinfection with a hot bead sterilizer. Xenopus skin is very sensitive to thermal damage, so it is imperative that the instruments are allowed to cool prior to using them after hot bead sterilization. Cold sterilization is not recommended due to the toxic nature of many of the chemical compounds and the permeable nature of Xenopus skin. (NIH, 2013)
2. No more than 5 animals should be used per pack of sterile instruments.
3. Use new clean or sterile gloves for each animal.
4. Clean the surgical area with an appropriate disinfectant between animals.

10. Post-Operative Recovery, Monitoring and Care

1. Animals must be visibly observed and monitored every 15 minutes during recovery from anesthesia until the animal is ambulatory and exhibiting species specific behaviors.
2. Because amphibians are highly resistant to hypoxia (oxygen deprivation), an amphibian should not be considered dead until prolonged attempts at resuscitation have been tried and rigor mortis has become apparent.
3. Appropriate Recovery Procedures:
   1. If utilizing immersion or topical anesthetics, after incisional closure, rinse the amphibian with sterile saline or clean water to remove any remaining anesthetic.
   2. During recovery, partially submerge the Xenopus in fresh water with the head and nares held above water or place in a closed but not airtight container with a moist paper towel on the bottom. Other methods are also acceptable if the skin is kept moist and relative humidity in the immediate environment maintained at x > 70%.
   3. DO NOT submerge aquatic amphibians in water until they are ambulatory and fully recovered from anesthesia as they may drown.
   4. Considering housing the animals in an area with an increased ambient temperature (72-85°F) to reduce the recovery period.
4. Post-Operative Documentation
   1. Tanks/enclosures containing animals that have undergone surgery must be labeled. Labs are to use ULAM acetate system (unless approved by the IACUC to use otherwise) and affix a yellow acetate with a Surgery Observation Sticker (SOS) to the tank. The label or acetate will be kept on the tank for at least 14 days or until skin sutures, if applicable, are removed, or the animal is euthanized; whichever is longer. At this period, the laboratory staff or designee must examine all post-surgical animals at least once a day.
   1. The date of surgery and end date of monitoring must be recorded on the sticker.
   2. Wound clips, staples, and skin sutures must be removed by 14 days after surgery unless described otherwise in the IACUC-approved protocol or as recommended by a ULAM veterinarian to necessitate incomplete wound healing. Adequate healing is described as apposed wound edges without signs of dehiscence, increased redness, discharge, odor or overt swelling.
2. Daily post-operative monitoring and health status of the animals must be recorded during the post-operative monitoring period and records must be maintained in the post-operative documentation.
   1. Attached to this document is the Amphibian Anesthetic, Surgical and Post-Operative Monitoring Record. Laboratory staff may use this template, or develop and use their own system/template.
2. Records must contain - at a minimum - the following information:
   1. The Principal Investigator name and Protocol
   2. The animal species, strain, and animal ID
   3. The surgeon(s) name(s), the date of surgery, and the surgical procedure
   4. The doses and routes of administration for all drugs administered (anesthetics, analgesics, etc.)
5. “For as needed analgesic therapy - If the animal is not showing clinical signs of pain, this must be documented in the record during the post-monitoring period (e.g., "No clinical signs of pain observed, analgesics not administered.")) Post-surgical notes on the animal's recovery, and observation notes that may include comments on animal condition, surgical site, drugs administered, etc.
4. Date of end of monitoring indicating such.
3. Records must be stored in the animal room until the end of the month of the post-operative monitoring period. Laboratory staff will place all surgical records (or copies thereof) at the drop box in the animal holding room. At the end of the month, husbandry staff clips all surgical records in the drop box and submits the compiled documents to the building husbandry supervisor who then purges the records after a year of maintenance.
4. For more information, please see ULAM Guidelines on Medical Records for Investigative Personnel.

5. Related Documents
1. Guidelines on Administration of Substances to Laboratory Animals
2. Guidelines on Medical Records for Investigative Personnel
3. Anesthesia and Sedation Monitoring Guidelines
4. Anesthesia and Analgesia Drug Descriptions
5. Amphibian Anesthetic, Surgical and Post-Operative Monitoring Record

6. Appendices

1. Appendix A: Parameters to Monitor Anesthetic Depth in Amphibians

<table>
<thead>
<tr>
<th>Reflex</th>
<th>Presence During Anesthetic Phase</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction</td>
<td>Light</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>Deep</td>
<td></td>
</tr>
<tr>
<td>Righting Reflex</td>
<td>Yes (slowed)</td>
<td>No</td>
</tr>
<tr>
<td>Abdominal Respiration</td>
<td>Yes</td>
<td>Occasional</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>In deeper anesthetic</td>
<td>planes, respiration will occur</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cutaneously for applicable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>species and this is sufficient</td>
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<tr>
<td></td>
<td>to maintain adequate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>oxygenation.</td>
<td></td>
</tr>
<tr>
<td>Corneal Reflex</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Gular Respiration</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>The gular region is the skin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>underlying the mandible and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>around the ventral neck.</td>
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</tr>
<tr>
<td></td>
<td>During inhalation phase, the</td>
<td></td>
</tr>
<tr>
<td></td>
<td>gular pouch is filled with</td>
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<tr>
<td></td>
<td>air and visible.</td>
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<tr>
<td>Withdrawal Reflex</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Elicited by touch when the</td>
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<tr>
<td></td>
<td>animal is under a light plane</td>
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</tr>
<tr>
<td></td>
<td>of anesthesia vs. elicited by</td>
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</tr>
<tr>
<td></td>
<td>a painful stimulus like a toe</td>
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</tr>
<tr>
<td></td>
<td>pinch when the animal is under</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a deep plane of anesthesia.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>It is eventually abolished</td>
<td></td>
</tr>
<tr>
<td></td>
<td>when the animal is on a very</td>
<td></td>
</tr>
<tr>
<td></td>
<td>deep plane of anesthesia.</td>
<td></td>
</tr>
<tr>
<td>Spontaneous movements</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cardiac Impulse</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>This is the ability to</td>
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</tr>
<tr>
<td></td>
<td>visualize the heartbeat</td>
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</tr>
<tr>
<td></td>
<td>through the skin overlying the</td>
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<tr>
<td></td>
<td>ceolomic cavity.</td>
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<tr>
<td></td>
<td>It becomes less obvious as the</td>
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</tr>
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<td></td>
<td>animal reaches deeper planes</td>
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<tr>
<td></td>
<td>of anesthesia.</td>
<td></td>
</tr>
</tbody>
</table>

2. Appendix B: Dosage Recommendations for MS-222 Administration in Amphibians

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosage and Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenopus - Adult</td>
<td>0.1 - 0.5% (1-5 g/L) immersion</td>
</tr>
<tr>
<td>Xenopus - Tadpole and larvae</td>
<td>0.01% -0.05% (0.1 - 0.5 g/L) immersion</td>
</tr>
<tr>
<td>Bufonids</td>
<td>0.2 - 0.3% (2-3 g/L) immersion</td>
</tr>
<tr>
<td></td>
<td>100 - 400 mg/kg intraceolomic</td>
</tr>
</tbody>
</table>
3. Appendix C: Published Analgesic Doses for Amphibian Species

<table>
<thead>
<tr>
<th>Drug and Class</th>
<th>Dose Range</th>
<th>Duration and Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine (opioid)</td>
<td>a. 14 mg/kg in the dorsal lymph sac b. 38 mg/kg SQ</td>
<td>a. Duration is variable b. x &gt; 4 hours in leopard frogs (Carpenter, 2013)</td>
</tr>
<tr>
<td>Butorphanol (opioid)</td>
<td>a. 25 mg/kg intracoelomic b. 0.2 - 0.4 mg/kg IM</td>
<td>a. 12 hours b. Efficacy uncertain (Carpenter, 2013)</td>
</tr>
<tr>
<td>Morphine (opioid)</td>
<td>a. 114 mg/kg in dorsal lymph sac b. 38-42 mg/kg SQ</td>
<td>a. 5 hours b. x &gt; 4 hours (Carpenter, 2013)</td>
</tr>
<tr>
<td>Flunixin meglumine (Banamine®, NSAID)</td>
<td>25 mg/kg intracoelomic</td>
<td>To be given once (Stevens, 2011)</td>
</tr>
<tr>
<td>Xylazine (alpha-agonist)</td>
<td>10 mg/kg intracoelomic</td>
<td>12-24 hours (Stevens, 2011)</td>
</tr>
<tr>
<td>Dexmedetomidine (alpha-agonist)</td>
<td>a. 120 mg/kg dorsal lymph sac b. 40 - 120 mg/kg SQ</td>
<td>a. 12-24 hours b. x &gt; 4 hours in leopard frogs (Carpenter, 2013)</td>
</tr>
</tbody>
</table>

*When pre-emptive analgesia is used, consider reducing the dose of anesthetic (whether inhalant or injectable) to the low end of the recommended range. Anesthetic depth must be carefully monitored and drug doses may need to be titrated to maintain appropriate levels. With new projects, sexes, strains or anesthetic analgesic combinations, assess a subset of animals before expanding to use in a larger cohort.

7. References