

## Surgery

### Item 5 – Guidelines for Anesthesia, Surgery and Analgesia in Amphibians

Anesthesia and aseptic surgery in amphibians presents a particular challenge. There are not many anesthetics and analgesics which have been adequately tested to ensure safety and efficacy in amphibians and the aquatic nature of these animals can make the preparation and maintenance of an aseptic surgical field very difficult.

The following guidelines are intended to provide the investigator with the DLAR-preferred procedures. This does not preclude the use of other agents/procedures, as long as they are approved by the Department of Laboratory Animal Resources (DLAR) veterinary staff and included in the approved Institutional Animal Care and Use Committee (IACUC) protocol prior to implementation.

All surgery must be performed or directly supervised by trained, experienced personnel. Inexperienced personnel must receive training in handling, anesthesia, and aseptic surgical techniques, which is available from the DLAR veterinary staff.

#### ANESTHESIA

##### General:

Amphibians begin life in an aquatic phase with the majority of their respiration occurring through gills. Most will eventually proceed through metamorphosis to become air breathing; whether or not they become terrestrial. It is important to remember that, although an amphibian may be completely aquatic, they still require air to breath and, if anesthetized by immersion, can drown if left submerged too long.

Whether they have undergone metamorphosis or not, amphibians also carry out a significant portion of their respiration through their skin. This high absorptive capacity of the skin is often exploited to provide anesthesia by immersion or other types of skin application.

Although not absolutely necessary, it may be useful to fast animals for 12-24 hours prior to anesthesia to prevent regurgitation. This can be especially useful if anesthetizing by immersion so as not to contaminate the anesthetic solution with stomach contents and/or high fecal output.

##### MS-222 (Tricaine Methanesulfonate):

**Dose: 250-2000 mg/L in buffered solution for immersion.**

MS-222 is the preferred anesthetic agent because of its ease of use and wide safety margin. Since all other agents are given parenterally or in high concentration at one point on the skin, the potential for overdose is very high and cannot be readily reversed. MS-222 is easily controlled by removal from the anesthetic solution and rinsing with clean water.

Solution should be made fresh daily and buffered to maintain a neutral pH. Sodium bicarbonate works well for buffering (400-1000 mg/L).

It may take up to 15 minutes to reach an adequate plane of anesthesia (as determined by loss of abdominal respiration and no response to a firm pinch on an extremity). If completely removed from the MS-222 solution, anesthesia will usually last between 10-80 minutes. This can be prolonged by either placing the animal in a shallow pool of anesthetic or wrapping the animal with gauze soaked with anesthetic. In either case, the prolongation anesthetic should be a low-concentration (250 mg/L) of MS-222.

### **Ice Bath (or other methods of rapid cooling):**

**Although traditionally believed to be an adequate anesthetic or adjunct to anesthesia, there is sufficient conflicting data within the literature as to bring its adequacy into question. Therefore, until this issue can be resolved, cooling of any kind will NOT be accepted unless adequate scientific justification for its use can be provided.**

Along with the question of anesthetic adequacy, there is also some concern that rapid cooling is stressful and/or painful for the animals.

And lastly, there is concern from an anesthetic standpoint that since cooling decreases the animal's metabolism, they may actually be metabolizing less anesthetic agent and therefore not receiving an adequate dose.

### **ASEPTIC PROCEDURES**

It is very important that the skin remain moist throughout the procedure. Not only is it damaging to the skin if it dries out, but cutaneous respiration will decrease and the animal may become hypoxic.

Skin away from the surgical site should be draped in moistened gauze and then covered with a clean drape to help maintain an aseptic surgical field.

The surgical site (as small as is practical) should be swabbed with an antiseptic solution such as chlorhexidine or povidone-iodine (Betadyne®). Do not use alcohol as this can be irritating to the skin and may be absorbed into circulation compounding the effects of the anesthetic agent(s).

Instruments should be sterilized before first use and either sterilized or rinsed in 70% ethanol (followed by sterile water rinse) between each animal. Gloves should be sterile before first use.

### **POSTSURGICAL CARE**

All animals must be monitored by trained personnel until completely recovered from anesthesia (displaying normal behavior and locomotion). This normally takes 30-90 minutes after removal of anesthetic agent.

Supplemental heating should not be provided as this will increase metabolic rate which will increase oxygen demands such that they may exceed the capacity of the cutaneous respiration leading to hypoxia.

### **ANALGESIA**

#### **Xylazine Hydrochloride:**

**Dose: 10 mg/kg intracoelomic every 24 hours.**

Due to its potential sedative properties, this should not be administered until the animal has completely recovered from surgical anesthesia.

## **RECORDS**

Appropriate records of all anesthesia administrations, surgical procedures and postoperative care must be maintained by the investigator. Although others (DLAR) may document observations on these records, responsibility for producing and maintaining these records lies with the Principle Investigator.

## **REFERENCE**

Anesthesia and Analgesia in Laboratory Animals, Academic Press, 1997. pp. 359-362.

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