



INDIANA UNIVERSITY
Office of Research Compliance (ORC)
Institutional Animal Care and Use Committee (IACUC)

Guidelines for Mouse Tail Biopsy

This guidance applies to all animals owned by IUSM for use in teaching or research on IACUC-approved protocols. The purpose of this guidance is to describe standard tail biopsy protocol and clarify when analgesia or anesthesia is required. The intent of this document is to define the IACUC's policy and to provide guidance to investigators.

Background

The *Guide for the Care and Use of Laboratory Animals* and Public Health Service Policy requires provision of pain relief for procedures which may cause pain or distress in animals.^{1,2} For the purposes of this policy, tail biopsy is defined as the removal of the distal portion of the tail.

Tail biopsy creates injury to a variety of tissues, including bone or cartilage, blood vessels, nervous tissue, and skin. Tail biopsy induces immediate pain which can last for 30-60 minutes³, and also has the potential to cause longer-term pain. The amount of tail tissue removed, strain/genotype, and animal's age impacts the degree of pain and discomfort associated with the procedure. The ideal age for tail biopsy in mice is between approximately 10-21 days of age. At this age the distal tail is not fully ossified in most mouse strains, making the procedure less painful and reducing likelihood of complications.⁴

Tail biopsy has historically been used for a variety of experimental reasons including blood collection and genotyping. Alternative procedures that do not involve removal of cartilage or bone should be considered as a refinement during study design. For example, molecular techniques such as PCR for genotyping can often be performed on saliva, hair, or tissue taken from ear notching. Several blood collection techniques which do not require tissue removal are available and are preferable to tail biopsy.

If tail biopsy must be used in adult animals for experimental reasons, investigators must include scientific justification in their IACUC protocol along with documentation for alternatives search from the literature. Repeated tail biopsy over the course of an animal's lifetime is strongly discouraged and also requires IACUC approval.

Statement

Tail biopsy must be described and scientifically justified in an approved IACUC protocol in order to be conducted on animals at IUSM. Any exceptions from the standard tail biopsy procedure outlined below must be reviewed and approved by the IACUC.

Standard Tail Biopsy Procedure at IUSM

- The amount of tail tissue to be removed will be limited to the smallest amount necessary to achieve the experimental goal. Total cumulative length of tail tissue removed will not exceed 5 mm per mouse over the course of an animal's lifetime.

- Aseptic technique will be used and includes use of sterile instruments and disinfection of the skin prior to biopsy. Instruments which will be re-used will be kept sharp and in good working condition.
- Following removal of tissue, hemostasis will be assured. Digital pressure, styptic powder, or silver nitrate will be used to assist with coagulation. Animals must be monitored as long as necessary by research staff to assure hemostasis is complete.
- Tail biopsy will be completed between 10-21 days of age AND less than 5 mm of tail tissue will be removed in total per mouse. When both of these two parameters are met, analgesia is recommended but not required.

Pain Relief Requirements for <i>Non-Standard</i> Tail Biopsy Procedures in Mice at IUSM						
Animal Age	Total Length Tail Removed Over Lifetime	Frequency of Biopsy	Local Analgesia Required	Systemic Analgesia Required	General Anesthesia Required	Analgesic and Anesthetic Agents
≤ 21 days	>5mm but ≤1cm ^b	Once or Multiple	Yes	Yes	No	Carprofen or Meloxicam or Ketoprofen at 5mg/kg SQ or Buprenorphine at 0.05 mg/kg SQ Before Biopsy <u>and</u> Topical 0.75% Bupivacaine Immersion for 30 Seconds Immediately After Biopsy
≤ 21 days	>1cm but ≤ 2cm ^c	Once or Multiple	Yes	Yes	Yes	Carprofen or Meloxicam or Ketoprofen at 5mg/kg SQ or Buprenorphine 0.05 mg/kg SQ Before Biopsy <u>and</u> General Anesthesia (Isoflurane) <u>and</u> Topical 0.75% Bupivacaine Immersion for 30 Seconds Immediately After Biopsy
>22 days	Up to 2cm ^d	Once or Multiple	Yes	Yes	Yes	Carprofen or Meloxicam or Ketoprofen at 5mg/kg SQ or Buprenorphine 0.05 mg/kg SQ Before Biopsy <u>and</u> General Anesthesia (Isoflurane) <u>and</u> Topical 0.75% Bupivacaine Immersion for 30 Seconds Immediately After Biopsy

- If tail biopsy is used as a blood collection method the IUSM Mouse Tail Biopsy policy will also apply for provision of analgesia and anesthesia. The use of this method more than once in the animal's lifetime is strongly discouraged. The PI must scientifically justify why other blood collection methods are not suitable.

Pain Relief Recommendation for Standard Tail Biopsy Procedure in Pre-Weanling Mice at IUSM						
Animal Age	Total Length Tail Removed Over Lifetime	Frequency of Biopsy	Local Analgesia Required	Systemic Analgesia Required	General Anesthesia Required	Suggested Analgesic Agent
< 21 days	Up to 5 mm ^a	Once or Multiple	No (Recommended)	No	No	Topical 0.75% Bupivacaine Immersion for 30 Seconds Immediately After Biopsy

^aTotal cumulative tail tissue removed will not exceed 5 mm (including any re-cuts).

Exceptions

Where exceptions to the standard tail biopsy procedure at IUSM are required for experimentally justified scientific reasons, the following table provides information on necessity of analgesia or anesthesia. Veterinarians are also available to provide guidance to investigators and can be reached by contacting the IACUC office at somiacuc@iupui.edu.

^bTotal cumulative tail tissue removed will not exceed 1cm (including any re-cuts).

^cTotal cumulative tail tissue removed will not exceed 2cm (including any re-cuts).

^dTotal cumulative tail tissue removed will not exceed 2cm (including any re-cuts).

Common types of analgesia/anesthesia used for tail biopsy:

Local Analgesics

Local analgesic agents are applied either prior to performing tail biopsy or directly to the cut tissue surface of the tail after biopsy and provide short-term (several minutes to ~1 hour) duration analgesia depending on the agent and method of administration. Onset of action varies with a number of factors but is generally considered immediate when applied on cut tissue surfaces and intermediate (~20-30 minutes) when applied to intact skin. Examples include lidocaine, bupivacaine, and benzocaine. Local analgesic agents are also referred to as local anesthetics. They are usually available in liquid or gel format.

Special Notes:

Ethyl chloride, ice-cold ethanol, and ice-cold isopropyl alcohol are no longer recommended for use on tail tips due to the potential for cryoinjury and lack of scientific evidence of significant analgesic effect in mouse tail biopsy.^{5,6} Ethyl chloride is carcinogenic and can be associated with tail tissue damage and necrosis.^{5,6,7}

Systemic Analgesics

Systemic analgesics are pain relievers which are administered either parenterally or orally. Systemic analgesics provide longer duration analgesia than local analgesic agents. They may be given alone or in combination with a local analgesic or general anesthetic. Onset of action requires approximately 20-30 minutes to allow for metabolism. Systemic analgesic agents fall into two main classes: non-steroidal anti-inflammatory agents (NSAIDs) and opioids. Examples of commonly used NSAIDs in mice include carprofen, meloxicam, and ketoprofen. Common examples of opioid drugs used in mice include buprenorphine, morphine, and butorphanol.

Special Notes:

- Opioid class drugs are classified as controlled substances/scheduled drugs due to their potential for abuse.
- Use of controlled substances in laboratory animals at IUSM requires a State of Indiana Controlled Substance Registration (CSR) and a federal DEA license.

General Anesthetics

General anesthetics are used to induce unconsciousness and a surgical plane of anesthesia. General anesthetics do not provide analgesia once the animal regains consciousness and so are usually paired with a local and/or systemic analgesic agent. Commonly used examples in mice include inhalant isoflurane and injectable ketamine cocktail-based formulations.

Special Notes:

Ketamine is a controlled substance and requires special licensing and handling requirements.

References

1. *Guide for the Care and Use of Laboratory Animals*, National Research Council, 8th Edition, pp 120-123.
2. Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002. Health Research Extension Act of 1985, Public Law 99-158, Animals In Research, Principle V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.
3. Jones, CP, Carver S, Kendall, LV. *Evaluation of Common Anesthetic and Analgesic Techniques for Tail Biopsy in Mice*. JAALAS 51 (6), Nov. 2012 pp 808-814.
4. Hankenson, FC, Garzel, LM, Fischer, DD, Nolan B, Hankenson, KD. *Evaluation of Tail Biopsy Collection in Laboratory Mice (Mus musculus): Vertebral Ossification, DNA Quantity, and Acute Behavioral Responses*. JAALAS 47 (6), Nov. 2008 pp10-18.
5. Braden, GC, Brice, AK, Hankenson, FC. *Adverse Effects of Vapocoolant and Topical Anesthesia for Tail Biopsy of Prewearling Mice*. JAALAS 54 (3), May 2015 pp 291-298.
6. Laboratory Animal Resource Center at IUSM, unpublished data (publication pending).
7. Hankenson, FC, Braden-Weiss, GC, Blendy, JA. *Behavioral and Activity Assessment of Laboratory Mice (Mus musculus) After Tail Biopsy Under Isoflurane Anesthesia*. JAALAS 50 (5), Sept. 2011 pp 686-694.

Contact

Please contact the School of Medicine IACUC office if you have any questions about this guidance.

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