



## Guidelines for Non-Survival (Terminal) and Survival Surgery in Rodents

Date of IACUC Review and Approval: October 4, 2019

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### I. General:

- This document serves as a supplement to the IACUC Rodent Surgery Policy. It is expected that any personnel conducting terminal (non-survival) or survival surgery in rodents is adherent to both the Policy and these Guidelines.
- The term survival surgery applies to any situation where surgical procedures are conducted and the animal recovers from anesthesia, regardless of the anesthetic time interval and how long until euthanasia. The use of good surgical technique to improve experimental outcomes is well established in the reduction of post-surgical complications, including infections, improved survival rates, and a more rapid return to basal physiological state. Anesthetic depth monitoring and post-operative monitoring is required for all survival surgeries.
- A non-survival (terminal) surgery is one in which the animal is euthanized during the procedure, while under a surgical plane of anesthesia. Anesthetic depth monitoring is required for any non-survival procedure expected to last longer than 15 minutes.
- This document is intended to assist principal investigators (PIs) performing surgical manipulations in rodent species and is consistent with those described in the *Guide for the Care and Use of Laboratory Animals* (The Guide), the *USDA Animal Welfare Act/Regulations* (AWA/AWR), and the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy).

### II. Procedures for Non-Survival (Terminal) Surgeries

- Preparation of the Surgical Field
  - A procedure room within the facility or a dedicated space in the lab may be used to conduct these surgeries. The area should be uncluttered and clean.
  - This area must be included in the IACUC approved protocol and will be inspected twice a year as part of the IACUC semi-annual inspection process.
- Preparation of the Patient and Surgeon
  - At a minimum, the surgical site should be clipped and the surgeon should wear gloves (as well as other PPE necessary for working with rodents).
  - More careful attention to aseptic technique may be necessary in the case of procedures of extended duration. Please see **III. Procedures for Survival Surgeries** for guidance.
- Preparation and Handling of the Instruments
  - Instruments for non-survival surgery do not need to be sterilized, but they should be clean and free of any debris.

- If multiple animals are done on a single day, the instruments need to be cleaned between animals.
- Intra-Operative Monitoring
  - Before beginning surgery, a firm toe pinch using either 2 fingers or atraumatic forceps must be done. Any reaction is an indication that the animal is not at a surgical plane of anesthesia.
  - Non-survival surgeries lasting 15 minutes or less in duration do not require further documentation of anesthetic monitoring.
  - For any procedures lasting longer than 15 minutes, anesthetic monitoring with documentation is required. This includes performing a toe pinch and assessing respiration, at a minimum, every 15 minutes. Although the procedure is non-survival, this ensures that the animal is being maintained at an appropriate anesthetic plane.
  - It is the responsibility of the surgeon to monitor respirations. A sudden noticeable increase in respiration rate may indicate that the animal is not deep enough and requires additional anesthesia, while a sudden noticeable decrease in respiratory rate may indicate that an animal is too deeply anesthetized.
  - Observation of the animal's color is another indication of anesthetic depth. The animal's ears, muzzle, and mucous membranes should be pink. If they are cyanotic (blue), there is poor oxygenation and this may indicate a respiratory issue. If they are pale, this indicates poor perfusion or blood loss, and may indicate a heart related issue.
- Surgical Record Keeping: The following information must be included in the surgical records.
  - PI name and protocol number
  - Title or brief description of procedure performed
  - Species, identification, and total number of animals
  - Name of the surgeon
  - Date of the surgery
  - Name and dose/dosage of all agents administered before and during anesthesia and/or surgery, including anesthetics, analgesics, therapeutics, and any experimental agents delivered
  - Monitoring of vital signs
  - Any complications (e.g. respiratory distress, bleeding, or unanticipated mortality) that occurred during the procedure
  - How the animal was euthanized at the end of the procedure, including primary and secondary methods

### III. Procedures for Survival Surgeries

- Preparation of the Surgical Field
  - A separate, dedicated operating room is not required to perform surgery in rodents. A facility procedure room or a space in the lab may be used. If surgeries will be done in the lab, it must be done in an area dedicated to surgery. The area should be clean, uncluttered, well organized, and relatively free from traffic. This must be described in the IACUC approved protocol and will be inspected twice a year as part of the IACUC semi-annual inspection process.
  - Before collecting the animal from the facility, gather and aseptically lay-out all of the needed items including: sterilized instruments, implants, suture material, anesthetics,

analgesics, etc. Set up your field with drapes, a warming device, and lighting prior to anesthetizing animals.

- Preparation of the Patient: Animal preparation must occur in a separate area from the operating space and after the animal is sedated/anesthetized. It includes the following:
  - Placing ophthalmic ointment in both eyes to prevent drying of the cornea during surgery.
  - Removal of hair/feathers from the surgical site. If using a cream hair removal, it is important to be sure to remove all of it prior to surgery to avoid chemical burns.
  - The shaved/plucked area should be wide enough to avoid contamination from the surrounding skin and hair/feathers during surgery, but should be the minimum compatible with achieving an appropriate sterile field. Removing more hair/feathers than is necessary predisposes the animal to hypothermia during the surgical procedure.
  - Hair/feathers that have been removed, along with hair removal cream if used, should be cleaned away using water and/or alcohol.
  - The animal can then be moved to the surgical field.
  - The skin of the planned surgical site should be disinfected using an appropriate surgical scrub pattern (in a concentric pattern moving from the innermost to outermost areas). This should be done with either dilute chlorhexidine or povidone iodine, alternated with sterile water or alcohol, and repeated three times.
  - The animal is then draped with the sterile surgical site the only part of the animal not covered. This can be done using paper drapes, reusable fabric drapes, or Glad Press 'N' Seal®.
  
- Preparation of the Surgeon and Surgical Assistant(s): Surgeons and any person who will have contact with the sterile surgical field during the surgery (surgical assistants) must prepare in the following manner:
  - They must wear the appropriate personal protective equipment (PPE) including a disposable gown (if in facility) or lab coat (if in lab), bouffant cap, mask, and booties.
  - The face mask and bouffant cap must be placed before donning sterile gloves.
  - The hands should be thoroughly washed and sterile surgical gloves of the correct size are then donned using appropriate technique so as to not contaminate the outside of the gloves.
  - Once the surgeon has donned sterile gloves, care must be taken to avoid touching non-sterile items such as the table, anesthetic equipment, lights, or scopes. Sterile gauze or autoclaved squares of tin foil can be placed over items that may need to be adjusted during the surgery (i.e. lights, microscopes, stereotaxic equipment, etc.).
  - If a non-sterile item/surface is accidentally touched, the surgeon must change their contaminated gloves for a new sterile pair.
  - Non-surgeons that will not have contact with the sterile field must wear appropriate PPE, which is at least a disposable gown (if in facility) or lab coat (if in lab), bouffant cap, mask, and booties.
  
- Preparation and Handling of the Instruments
  - All surgical instruments must be cleaned and autoclaved prior to use on animals for all survival surgical procedures.
    - Autoclaved surgical packs should contain a sterilization indicator, the date of sterilization, and expiration date for the pack.
    - Checking the indicator/mark on packaged instruments and checking that the outer packaging has not been breached will ensure that the sterilization process

- has been effective.
  - Equipment or implants that will come into contact with the sterile field that are unable to withstand the conditions of autoclaving must be sterilized in another way.
    - Ethylene oxide sterilization is available through ACF for a fee. It is important to plan ahead for the time it takes for processing and subsequent off-gassing.
    - Cold sterilization should only be used as a last resort when other methods are not appropriate. A specific cold sterilant must be used and the manufacturer instructions must be strictly followed. The instruments must be rinsed with sterile water or sterile saline before putting them in contact with animals.
  - If surgeries will be completed on multiple animals in the same session, a hot bead sterilizer can be used to sterilize the instruments between animals. It is important to use the hot bead sterilizer in the correct way to ensure sterilization.
    - It must be turned on and given sufficient time to heat up.
    - All blood and tissue must be cleaned from the instruments – do not use volatile compounds, such as alcohol.
    - The tips of the instruments should be placed at least 1.5 inches into the beads and left in place for at least 15 seconds.
    - Only 1-2 instruments should be sterilized at a time.
    - It is critical that the instruments are allowed to cool off before coming into contact with an animal to avoid severe, life-threatening burns.
  - All consumables that will be used during the procedure (e.g. gauze, swabs, needles, suture materials) must be sterile and should be of an appropriate size and packaged in suitable quantities.
  - Instruments should be placed on a sterile surface (e.g. sterile drape) when not being used.
    - Where it is suspected that instruments may have been accidentally contaminated, these must be replaced with sterile ones before continuing.
- Intra-Operative Monitoring
  - Animals must be monitored throughout the duration of the surgery to ensure an adequate plane of anesthesia. This must be done and documented every 15 minutes.
  - Before beginning surgery, a firm toe pinch using either 2 fingers or atraumatic forceps must be done. Any reaction is an indication that the animal is not at a surgical plane of anesthesia.
  - It is the responsibility of the surgeon to monitor respirations. A sudden noticeable increase in respiration rate may indicate that the animal is not deep enough and requires additional anesthesia, while a sudden noticeable decrease in respiratory rate may indicate that an animal is too deeply anesthetized.
  - Observation of the animal's color is another indication of anesthetic depth. The animal's ears, muzzle, and mucous membranes should be pink. If they are cyanotic (blue), there is poor oxygenation and this may indicate a respiratory issue. If they are pale, this indicates poor perfusion or blood loss, and may indicate a heart related issue.
- Surgical Record Keeping: The following information must be included in the surgical records.
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  - Species, identification, and total number of animals
  - Name of the surgeon
  - Date of the surgery

- Name and dose/dosage of all agents administered before, during, and after anesthesia and/or surgery, including anesthetics, analgesics, therapeutics, and any experimental agents delivered
  - Monitoring of vital signs
  - Any complications (e.g. respiratory distress, bleeding, prolonged recovery or unanticipated mortality) that occurred during or after the procedure
- Post-Operative Care
    - Close surgical wounds using appropriate techniques and materials. Proper wound closure is essential to avoid wound dehiscence.
    - Move the animal to a warm, clean cage. The animal's face should be placed on a paper towel, or something similar, to avoid the nose becoming buried in the bedding, as this can lead to asphyxiation. The cage should be placed half on and half off of a warming device.
    - Avoid placing recovering rodents in a cage with awake animals to avoid cannibalistic behavior and injury to the anesthetized animal.
    - The animal must be continuously monitored until fully recovered from anesthesia. Once recovered, indicated by normal ambulation and behaviors, it can be returned to its normal housing location.
    - Provide analgesics as approved in your IACUC protocol.
    - Typically, skin sutures and wound clips are removed 10-14 days post-operatively, once the incision is completely healed.
    - Animals must be monitored post-operatively as outlined in the IACUC-approved protocol. For survival surgeries, this is typically twice a day for at least 3 days following surgery.
    - Complete and place a Surgical Care card on the cage. These should be available in all rodent rooms.

<b>SURGICAL CARE</b>	
PI:	_____
IACUC #:	_____
Sub PI:	_____
Procedure Performed:	_____
Date/Time:	_____
Analgesics:	_____
Date/Time:	_____
<b>Post-op Checks:</b> (use optional)	
Date/Time:	_____ Initial: _____
Date/Time:	_____ Initial: _____
Date/Time:	_____ Initial: _____
Date/Time:	_____ Initial: _____
Date/Time:	_____ Initial: _____
Date/Time:	_____ Initial: _____
Special Directions:	_____
_____	

- Post-Operative Record Keeping: The following information must be included in the post-operative monitoring records.
  - Monitoring the integrity of any surgical incisions, ensuring that they are clean, dry, and intact.
  - The animal's general posture and activity.
  - Hydration status and appetite – urine and feces should be present in the cage and indicate that hydration and appetite are normal. A hydrated mouse has sunken eyes and may have pale ears.
  - Additional comments for any variations from the normal and expected events during the recovery period.
  - The animal's weight must be recorded if it's included in the IACUC protocol (this is generally the most objective measure of well-being).
  - Analgesics used including the time, dosage, and route.
  - The initials of the person doing the monitoring.
  - The frequency of monitoring must adhere to what is described in your approved IACUC protocol.
  - **Note:** The length of monitoring noted in the protocol is a minimum. If there is any indication that the animal is not doing well, the record must extend beyond this period.

**IV. Record Keeping:** Records documenting any non-survival surgery (terminal), survival surgery, and post-operative monitoring must be retained by the lab and readily available for review by the IACUC, the veterinary staff, and representative of regulatory and accrediting organizations. All rodent anesthesia and surgery records must be retained for a minimum of one (1) year. The following

appendices are attached to use as-is or as a starting point for the lab to develop their own, procedure-specific form.

- Appendix 1: Rodent Anesthesia Monitoring Record (Non-Survival Surgery)
- Appendix 2: Rodent Anesthesia Monitoring Record (Survival Surgery)
- Appendix 3: Rodent Post-Surgical Monitoring Record

## **V. References**

- NRC (National Research Council), Institute of Laboratory Animal Resources, Commission on Life Sciences. 2011. Guide for the Care and Use of Laboratory Animals, Washington D.C., National Academy Press.
- National Institutes of Health, Office of Laboratory Animal Welfare. 2015. PHS Policy on the Humane Care and Use of Laboratory Animals.
- The Animal Welfare Act, 7USC Sec 2131 et seq. Title 9, Code of Federal Regulations, parts 1, 2, & 3, January 1998.



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### Rodent Anesthesia Monitoring Record (Non-Survival Surgery)

Date:	PI:	Animal Protocol#:	Tag#/ID(s):	Species:	Strain:	Sex:
Procedure:		Surgeon:		Contact #:		
Anesthetic Agent(s): Dose (mg), route		Isoflurane _____% in oxygen		Time of Induction:		
Duration _____ hours	Surgical notes and/or complications:					

#### Surgical Monitoring

Animal Tag#/ID	Time	Negative Toe Pinch	Normal Breathing	Other (describe)	Animal Tag#/ID	Time	Negative Toe Pinch	Normal Breathing	Other (describe)	Animal Tag#/ID	Time	Negative Toe Pinch	Normal Breathing	Other (describe)
	:15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	:30	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:30	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:30	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	:45	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:45	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:45	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	:00	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:00	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:00	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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<p style="text-align: center;"><b>Post-Surgical Euthanasia</b></p> <p>(Method, dose, route as appropriate)</p>	
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**Rodent Anesthesia Monitoring Record (Survival Surgery)**

Date:	PI:	Animal Protocol#:	Tag#/ID(s):	Species:	Strain:	Sex:
Procedure:		Surgeon:	Contact #:	Pre-Surgical Weight (g):		
Anesthetic Agent(s): Dose (mg), route		Isoflurane _____% in oxygen	Fluids: Dose (mLs), route, type			
Time of Induction:	Time of Recovery:		Duration _____ hours			
Surgical notes and/or complications:			Pre-Surgical Analgesics (agent, dose, route):			
			Post-Surgical Analgesics:			

**Surgical Monitoring**

Animal Tag#/ID	Time	Negative Toe Pinch	Normal Breathing	Other (describe)	Animal Tag#/ID	Time	Negative Toe Pinch	Normal Breathing	Other (describe)	Animal Tag#/ID	Time	Negative Toe Pinch	Normal Breathing	Other (describe)
	:15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		:15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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