

	<b>Flinders University</b> <b>Safe Work Method Statement</b> <b>Necropsy of Rodents for Animal Welfare Investigations 18/06/19</b>			
				<b>College of Medicine and Public Health Animal Facility</b>
<b>SWMS Number</b>	<b>RA Number</b>		<b>AWC Approval Date</b>	
SWMS-	RA-		18/06/2019	
<b>Contact Person</b>	<b>SWMS prepared by</b>		<b>Review Date</b>	
AWO	Lewis Vaughan		June 2021	

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### Legislation

- *Australian Code for the Care and Use of Animals for Scientific Purposes 8<sup>th</sup> Ed.*
- *Animal Welfare Act 1985*
- *Animal Welfare Regulations 2012*
- [Gene Technology Act 2000](#) (the Act)
- [Gene Technology Regulations 2001](#)
- *Work Health and Safety Regulations 2012*

### University Policy

- Work Health and Safety Policy 2013
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

### Local Policy

Use of the College of Medicine and Public Health Animal Facilities by all staff and researchers of the College of Medicine and Public Health, Flinders University, is subject to awareness of, and adherence to the following:

#### Research Involving Animals:

- The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must submit a proposal to the Animal Welfare Committee (via the Animal Ethics Review Sub-Committee. No work with animals may commence until written approval has been received from the Animal Welfare Committee. Standardised application forms for Research and Teaching can be found on the Flinders University website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc.  
[http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics\\_home.cfm](http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm)

- **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).**

### Safe Work Method Statement

Refer to Risk assessments, Safe Work Method Statement for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager

SWMS 1.0	Mouse- Sexing, Handling, Restraint and Ear Notching
RA 1.0	Mouse- Sexing, Handling, Restraint and Ear Notching
SWMS 1.1	Mouse- Injection techniques
RA 1.1	Mouse- Injection techniques
SWMS 7.0	Compliance - Emergency Contingency
RA 7.0	Compliance - Emergency Contingency
SWMS 7.1	Compliance -Transportation
RA 7.1	Compliance -Transportation
SWMS 7.2	Rodent Importation
RA 7.2	Rodent Importation
SWMS 1.4	Methods of humane euthanasia of mice
SWMS 2.5	methods of humane euthanasia of rats

### Personal Protective Equipment Required

- **Gloves**
- **Gown**
- **Mask**
- **Hair Net**
- **Shoe Covers**
- **Goggles**

For PC2 animals, PPE must comply with PC2 requirements. However, PPE is recommended for PC1 animals to reduce risk of exposure to pathogens.

### Hazards and Controls

- **Needle Stick- DO NOT recap needles, dispose immediately into sharps containers, adhere to SWMS.**
- **Chemical exposure- avoid exposure, wear PPE and goggles.**

### Before Work Commences

**Ensure that you are aware of the locations of the following:**

- **Spill Kit**
- **Fire Extinguisher**
- **Eye Wash**
- **Exits**

**Risk Assessment and SDS (Safety Data Sheet) - Ensure that you have read and understood relevant documents for all substances used.**

### **Equipment**

- **Check for safety and electrical compliance**
- **Ensure that you have read and understood the Risk Assessment and Safe Work Method Statement**
- **Obtain training before using any equipment**
- **All procedures are to be performed by trained competent staff.**
- **Training is available from senior animal house staff or the Animal Welfare Officer.**
- **Evidence of training is available in the “Staff Training Needs Analysis” and records of competency assessment is available on the Animal Welfare Competency Skills Register**

### Equipment

- **Post mortem kit (large for rats, small for mice) – this must be sterile if samples for culture are required.**
- **70% ethanol.**
- **Scalpel blade if required and sharps container.**
- **Bag to contain carcass at the end of the procedure.**
- **Labels for outside of bag and pots.**

- **Plastic pots containing 10% buffered formalin.**
- **Tran swab for microbiological sample collection.**
- **Camera.**
- **Necropsy form and pen/pencil.**

### Location of Necropsy

**\*\*\*NOTE:** As Flinders University animal holding rooms are quarantine facilities, no animal is to be taken off site after death for necropsy without prior permission of the AWC Chair and IBC Chair.\*\*\*

- Animals housed in PC1 may be necropsied in the theatre, or in a room with a fume hood if formalin is used.
- Animals housed in PC2 must be necropsied in the biological safety cabinet in the PC2 room where they were housed.

### General Information

- A standardised approach to the necropsy of an animal may assist in the identification of the cause of death. Prompt necropsy may mean that there will be less autolysis during storage. Keeping samples in 10% buffered formalin can be used to preserve tissues for either pathology referral or inspection by the AWO. (NOTE: The maximum thickness of tissues for formalin fixation is 1 cm. The ratio of tissue to the volume of formalin is 1:10.) Please ensure that you follow safety precautions when using formalin. Always work in a fume cabinet and avoid skin contact with the agent. If histology is not required, and this is directed by the AWO, entire carcasses may be frozen for later welfare examination.
- Photographs taken during the post mortem examination are useful records. Digital photos should be added on the pages following the post-mortem examination form. The Animal Welfare Committee encourages the use of photographic records.

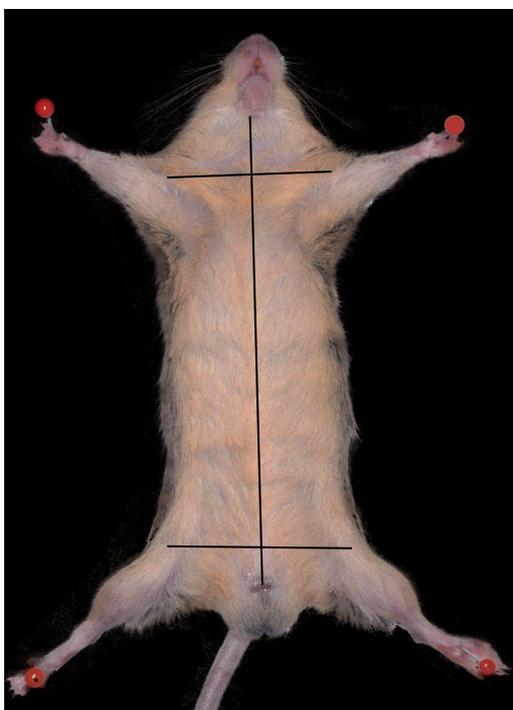
### External Examination and Preparation

1. Obtain a Rodent Necropsy Form (*Appendix 1*) and record information as the examination progresses.
2. Confirm the sex of the animal.
3. Record animal identification (ear notch, microchip, or colour).
4. Record bodyweight.
5. Determine a body condition score (1 is emaciated, 3 is a normal condition for animal's age and sex; 5 are obese). Each number reflects a 10-15% change from the 'normal' weight.
6. Inspect the coat (look for signs such as fur loss and ruffled coat).
7. Check the colour of the underlying skin (note signs of skin or tissue discoloration – tissues exhibiting dark bluish or bright red colours may be consistent with sepsis).
8. If tumours are present, record dimensions, location, whether discrete or diffuse, and consistency (soft, firm or hard).

9. Look for discharges from body orifices.
10. Record observations and photograph any visible abnormalities, if indicated.
11. If inflammation or sepsis is suspected, commence the post-mortem examination using aseptic technique until samples for culture are collected.

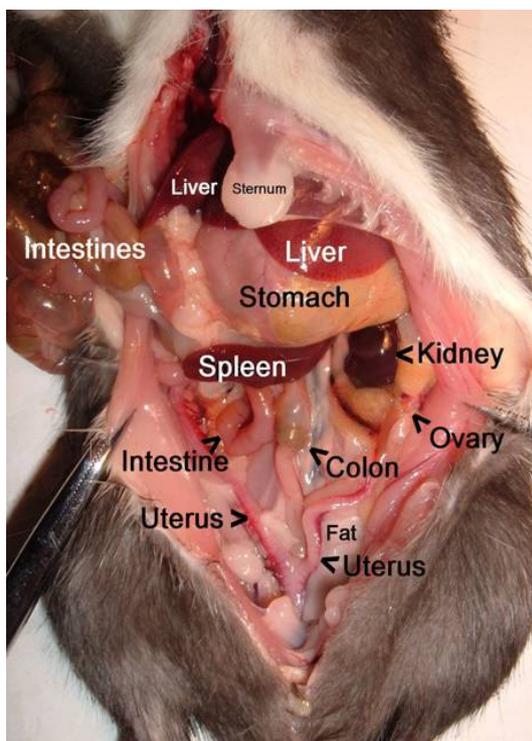
### Internal Examination

1. Place the rodent in dorsal recumbency on an impermeable surface, such as a benchcote, newspaper, plastic, or dedicated necropsy board.
2. Wet the rodent's coat with 70% ethanol. This is to wet the fur so it does not contaminate the necropsy.
3. Lift the skin with forceps and use scissors to make a full-length ventral midline longitudinal incision through the skin from the chin to the anus (*Figure 1*).



**Figure 1**

4. A generous incision made perpendicular to the midline along the legs will assist with the carcass sitting flat. Retract the skin.
5. If subcutaneous lesions that show evidence of bright red or bluish discoloration, swab these with a transwab or collect the tissue and place it into a sterile yellow top container using aseptic technique.
6. Incise the abdominal midline without perforating underlying viscera. Cut through the diaphragm and separate it from the costal arch of the last ribs.
7. The dorsal aspect of the ribs should be cut, and the rib cage removed, to expose the thoracic contents.
8. All thoracic and abdominal viscera are now exposed from the chest to the pelvis (*Figure 2*). If anomalies are found, take an image to include the chest and abdomen.



**Figure 2**

9. If samples for culture of viscera are required, use a transwab to swab tissues, or excise tissues now, using aseptic technique prior to contamination. Place the tissues for culture in a sterile yellow top container. Signs consistent with inflammation and infection are redness, or blueness and swelling.
10. Remove the respiratory tract from the thorax. Cut the side of the mouth down the neck and the trachea will be exposed. Lift the trachea and gently snip attachments away. As you lift the trachea, the lungs and heart can be lifted out of the body.
11. Cut the oesophagus where it enters the abdomen.
12. Place the heart and lung tissues aside. Look at the lungs from the dorsal surface (i.e. normal position if rodent was standing).
  - a. Normal lungs appear an even light pink (salmon in colour).
  - b. Congestion is a darker red/purple and may be seen with anaesthetic deaths.
  - c. Tumours may be visible on the external surface.
  - d. Cut into the lungs as if slicing bread (they should spring back into position). If they sag, this may be a sign of congestion.
  - e. Lung consolidation may be ascertained by the 'lungs float test'. Remove the lungs and place them in a beaker of water. If the organ sinks, consolidation may be present.
  - f. If indicated, place heart and lungs into 10% buffered formalin.
13. Grasp the oesophagus and use it to gently lift the stomach, liver, and intestines out of the abdominal cavity. Trim attachments with scissors to help lift the organs out. Cut the large colon where it enters into the pelvis. Remove the abdominal organs.

- a. The liver should be dark red or maroon. Check surfaces for changes in colour, which may represent an abscess or tumour. If indicated, place the liver in 10% buffered formalin.
  - b. The spleen sits on the greater curvature of the stomach. It should be a dark red colour, similar to the liver, not light pink. If indicated, excise the spleen and place into 10% buffered formalin.
  - c. The pancreas lies between the stomach and small intestine. It is light pink in colour.
  - d. The intestines can be teased out to check if there is a local lesion. Look for:
    - i. Purple areas or bright red = abnormal;
    - ii. Gas bubbles (few are normal, many are abnormal); or
    - iii. Presence of ingesta and digesta.
  - e. If indicated, the intestines can be placed in 10% buffered formalin.
14. If indicated, take an image of the remaining organs inside the body (kidney, reproductive, blood vessels, and lymph nodes).
- a. Note the appearance of the adrenal glands, which sit cranially and closer to the midline in relation to the kidney.
  - b. Remove a kidney and cut along its long axis. If indicated, place into 10% buffered formalin.
  - c. Ovaries sit behind the kidney (they may be small in non-gravid animals). Small yellow to white nodules in the ovary reflect breeding activity.
  - d. An ovary and uterus horn can be placed in 10% buffered formalin.
15. Take the scissors and cut into the corner of the mouth. Cut through the bone and then into the soft tissue. Flip the jaw to one side. At this point, the opening of the windpipe (larynx) should be visible.
- a. Check the larynx for saliva, blood, or food that may be obstructing it.
  - b. Check the teeth (they should be well aligned and be firm inside the sockets).
  - c. Cut down the length of the trachea (look for the presence of saliva, blood, or food).

### Removal of the Brain

1. This may not need to be done unless there is a reason for examining the brain (difficulty moving, seizures, circling, etc).
2. Turn the rodent over onto its belly.
3. With a scissors, cut the skin away from the top of the skull.
4. Cut down through the muscles at the back of the head to find the foramen magnum (*Figure 3*).
5. With fine scissors, cut from the foramen magnum to the top of the skull and lift off the brain.

6. The brain can be gently teased out of the skull cavity by tipping the rodent head upside down so that the brain begins to gently fall out. Scissors are used to cut the meninges and cranial nerves. If indicated, place the brain into 10% buffered formalin.



**Figure 3**

#### At Conclusion of Necropsy

1. Check that you have filled out a comment for any anomaly found in each organ on the necropsy form. The necropsy form is forwarded to the Animal Ethics Officer, along with images taken. Samples should be left with the facility manager or senior animal technician.
2. Once complete, the carcass is placed in a yellow plastic bag and marked for either autoclaving or incineration. Place the animal back into the cold room.
3. Ensure that the workspace has been cleaned and disinfected. Prepare necropsy kit for sterilisation.

#### Rodent Necropsy Template

## **Rodent Necropsy Template EXAMPLE**

**NEXT PAGE**

- The Rodent Necropsy Template can be download from the AWC website ([http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics\\_home.cfm](http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm)).

## SWMS Review

This SWMS currently applies to the animals housed in the College of Medicine and Public Health Animal Facility. This SWMS will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change.

Position	Name	Contact Details
Manager Animal Facility	Roxanne Collingwood	8204 4380 <a href="mailto:roxanne.collingwood@flinders.edu.au">roxanne.collingwood@flinders.edu.au</a>
Animal Welfare Officer	Lewis Vaughan	0450 424 143 <a href="mailto:awo@flinders.edu.au">awo@flinders.edu.au</a>

### **Useful References**

<http://www.nhmrc.gov.au>

<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/home-1>

<http://www.adelaide.edu.au/ANZCCART/>

[http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics\\_home.cfm](http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm)

[http://www.medipoint.com/html/for\\_use\\_on\\_mice.html](http://www.medipoint.com/html/for_use_on_mice.html)

Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facility Manager.

**FLINDERS UNIVERSITY / SOUTHERN ADELAIDE LOCAL HEALTH NETWORK  
ANIMAL WELFARE COMMITTEE**

## Post-Mortem Examination Report

Date of Death		Project Number	
Date of Post-Mortem Examination		Title of Project	
Researcher		Person Performing Necropsy	
Strain		Identification	
Weight (g)		Sex ( <i>Tick</i> )	<input type="checkbox"/> Male <input type="checkbox"/> Female
History ( <i>Events Leading Up to Death</i> )			

### External Examination

Organ	No Abnormality ( <i>Tick</i> )	Abnormality ( <i>Tick</i> )	Abnormal – Note Findings
Mouth & Nostrils			
Anus			
Coat and Limbs			
Body Condition Score (1-5)			

### Internal Examination

Organ	No Abnormality ( <i>Tick</i> )	Abnormality ( <i>Tick</i> )	Abnormal – Note Findings
Salivary Glands			
Cervical Lymph Nodes			
Inguinal Lymph Nodes			
Mammary Glands			
Liver			
Spleen			
Intestines (Small)			
Intestines (Large)			
Stomach			
Pancreas			
Mesenteric Lymph Nodes			
Kidneys & Ureter			
Adrenal Glands			
Bladder			
Male: Testis & Epididymis			
Female: Ovary & Uterus			

Eye			
Mouth			
Tongue			
Larynx			
Trachea			
Lungs			
Heart			
Brain			

### Summary of Outcome of Post-Mortem Examination

Macroscopic Findings	
Outcome of Examination	
Images Taken (Y/N)	
Samples Collected	
Follow Up Investigations	
Follow Up Actions Recommended	