

Rodent Euthanasia Guidelines and Recommendations



Research
*Division of Laboratory
Animal Resources*



Euthanasia Guidelines

In their 2020 report, the *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* reviewed the current literature and concluded that “the practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO₂, is unacceptable” and mandated the gradual displacement method of 30-70% CO₂ euthanasia as the only acceptable method.

For complete information, please refer to the full text of the UK IACUC Policies Guidelines, and Procedures 103 found on the [IACUC website](#)



Euthanasia Guidelines

Observe the procedure during a one-on-one training session before doing it yourself

Keep the animal as calm as possible

Maintain a professional demeanor; keep your focus on the animal

Make sure the procedure is performed correctly and accomplished humanely

NEVER perform euthanasia in an animal housing room



In All Cases, Death Must Be Ensured

By allowing a sufficient period of time in a chamber when using inhalant gases

By utilizing an unequivocal secondary means of ensuring death (decapitation, opening of the thoracic cavity) ***This is a required procedure when using CO₂***

What to Expect

The animal must **lose consciousness first**

Then motor function is lost

Animals may continue to have random reflex motor activity after losing consciousness

This is NOT evidence of pain or distress.



Inhalant Agents

Cause death by indirect hypoxia; anesthesia first, then hypoxia

Safety issues: can also be breathed by the technician!

Use proper concentrations

Maintain equipment properly

Do not use CO₂ as sole method in neonates– they are very resistant to hypoxia



Carbon Dioxide (CO₂)

Is Rapid, Effective, Easy to Use, Inexpensive, and Safe

Correct concentration is important to minimize distress in the animals:

- *100% CO₂ at a flow rate of 30-70% of chamber volume per minute

- *CO₂ must be supplied from either gas cylinders or building CO₂ gas distribution systems equipped with an appropriate pressure reducing regulator and flow meter combination (or equivalent) to permit precise regulation of gas flow to the chamber



How Many Mice/Rats Can I Put In The Cage

Mice: If you are grouping animals, you should not put more than 8-12 if they are weanlings and no more than 8 adults

If you can't see the floor and the animals can't move around, you have too many in the cage

Weanling don't have a problem with grouping nor do most females, but any adult males regardless of the strain will become aggressive

The best thing is to not combine cages unless you absolutely have to

Rats: No more than 4-5 depending on size, age, or weight if adults

No more that 10-12 if weanlings (less than 40 days of age)



Steps for Using Carbon Dioxide for Euthanasia

Place animals in appropriate chamber

Place top securely on cage

Turn CO₂ tank on

Introduce 100% carbon dioxide at a rate of 30-70% of the chamber volume per minute

Adjust flow meter to appropriate setting for cage size according to chart located above tank

Allow time for animal(s) to become unconscious and for all motion to cease



Carbon Dioxide Method

Do Not pre-fill the chamber

Use correct setting

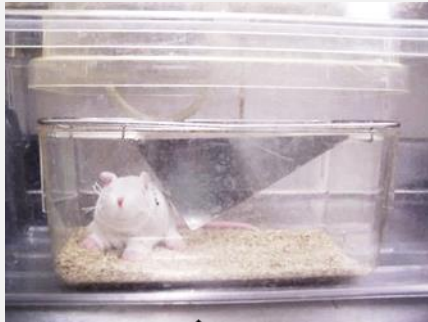
Consult wall chart for proper settings

Remember to turn off gas at main tank when finished



Carbon Dioxide Method

When placing mouse cages INSIDE the large cage to euthanize, be sure to set flow meter for mice to **5-7 liters per minute**. **Rats 8 up to 12 liters/min**



↑
Static micro-isolator **small** cage **3-4** liters per min.



Small mouse IVC **inside large** cage **5-7** liters per min.



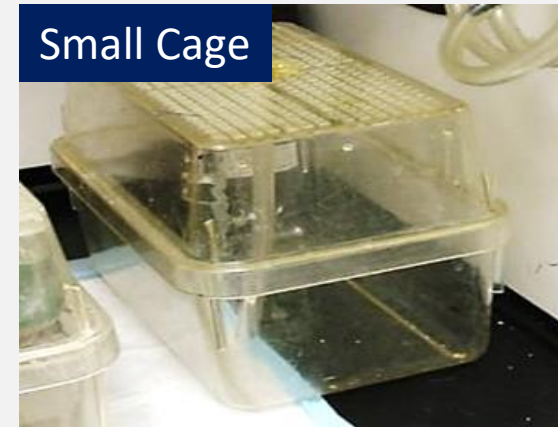
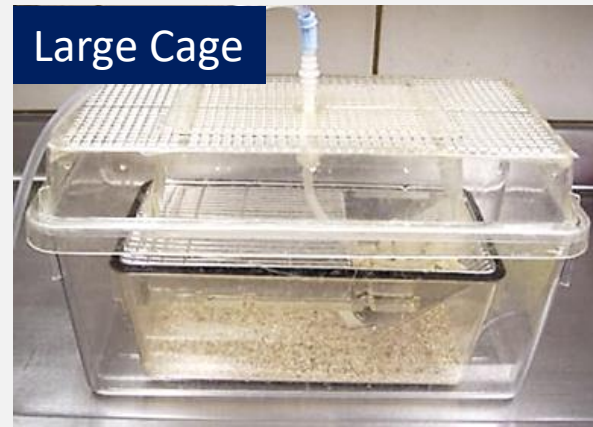
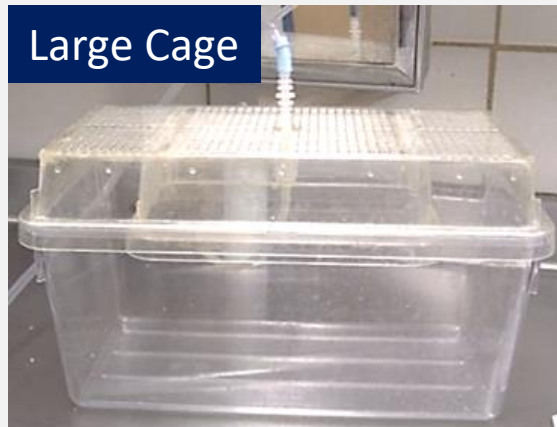
Large mouse IVC inside large cage **5-7** liters per min. **Rats 8 up to 12 liter/min**



Flow Rates for Carbon Dioxide

It is strongly recommended that animals stay in their home cage whenever possible. It is less stressful for the animals. All sizes of mouse cages fit in the large cage. Be sure to leave the wire bar lid in place.

Cage Type	Cage Size (W x L x H)	Flow Rate per IACUC Policy 103
Small (Mouse cage)	7.5" x 11.75" x 5"	For mice 3-4 liters/min For Rats 8 up to 12 liters/min
Large (Rat cage)	10.5" x 19" x 8"	For mice 5-7 liters/min For rats regardless of cage size 8 Up to 12 liters/min



You must remain with the animal during the procedure

Carbon Dioxide Method

Maintain gas flow for 1 minute after apparent death

Turn off CO₂ tank and allow animals to remain in unopened chamber undisturbed for several minutes to ensure that death has occurred

Ensure death by secondary method (cervical dislocation, pneumothorax, or decapitation for neonates is required)

The secondary method to ensure death is an IACUC requirement when using CO₂ is your method of euthanasia.

Required Secondary Methods to Ensure Death When Using Carbon Dioxide

Decapitation (pups)

Thoracotomy: open the chest to prevent breathing *

Exsanguination (bleed out)

Cervical dislocation

* When collecting tissue or major organs, the opening of the major body cavity and subsequent thoracotomy (disruption of the diaphragm) can serve as your secondary method.

Decapitation of Neonate Mouse Pups with Scissors

After the pups have been euthanized with Carbon Dioxide, the secondary means of euthanasia is decapitation for pups (0-10 Days Old).

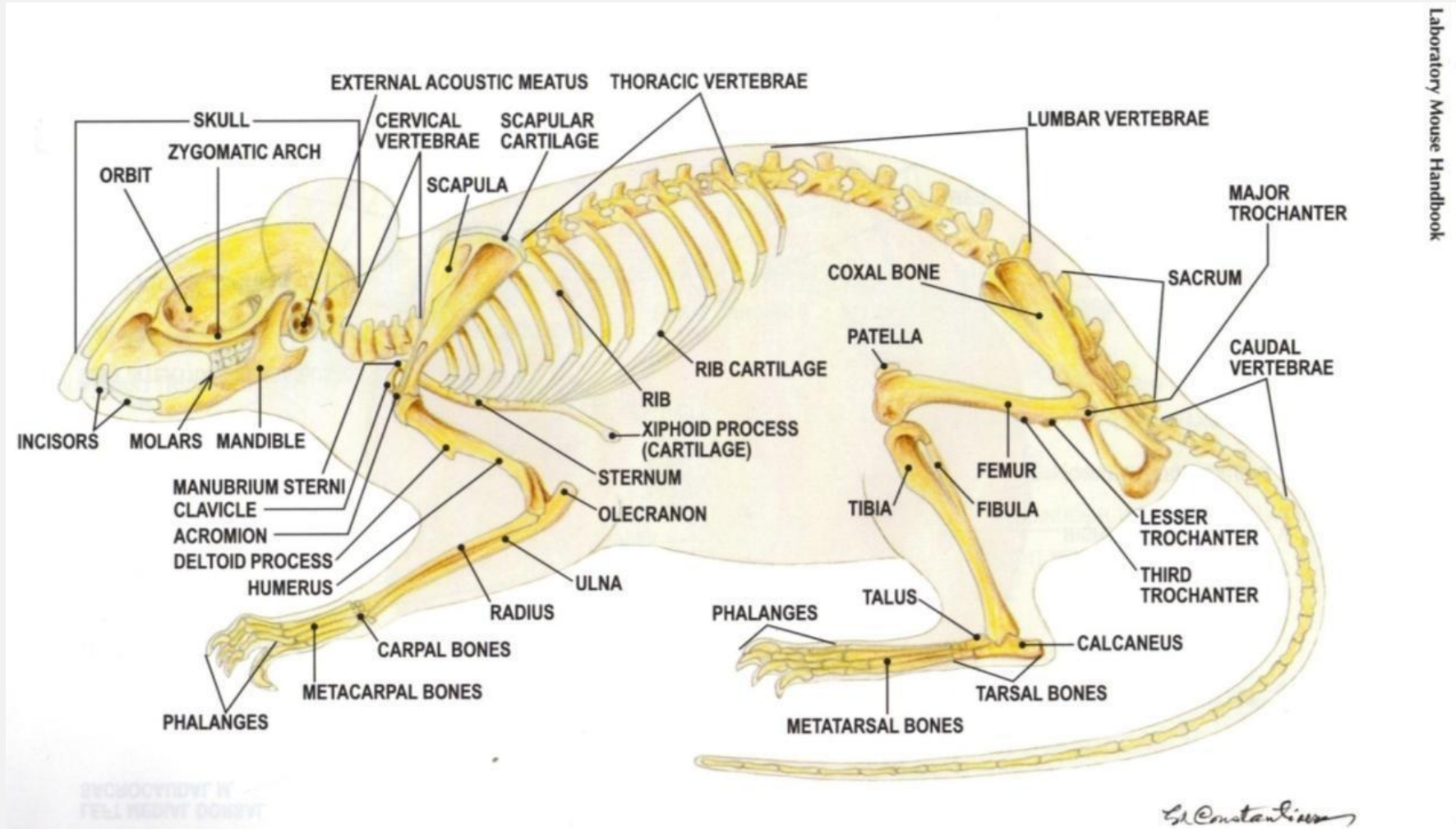
Take a **SHARP** pair of scissors and cut between the ears and the point of the shoulders



Cut Here

Cervical Dislocation in Mice and Rats as a Secondary Means of Ensuring Death

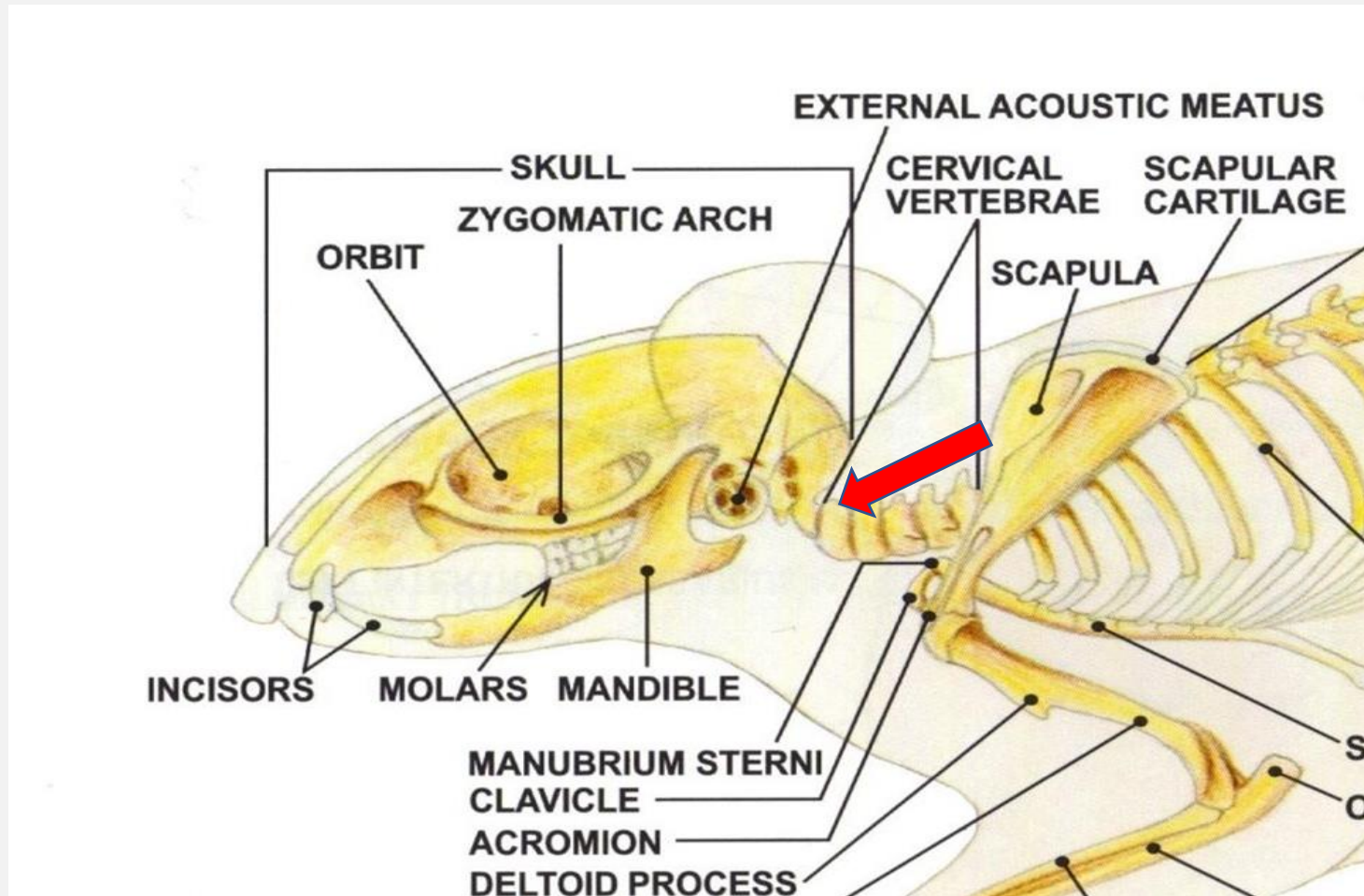
Skeletal Anatomy of the Mouse



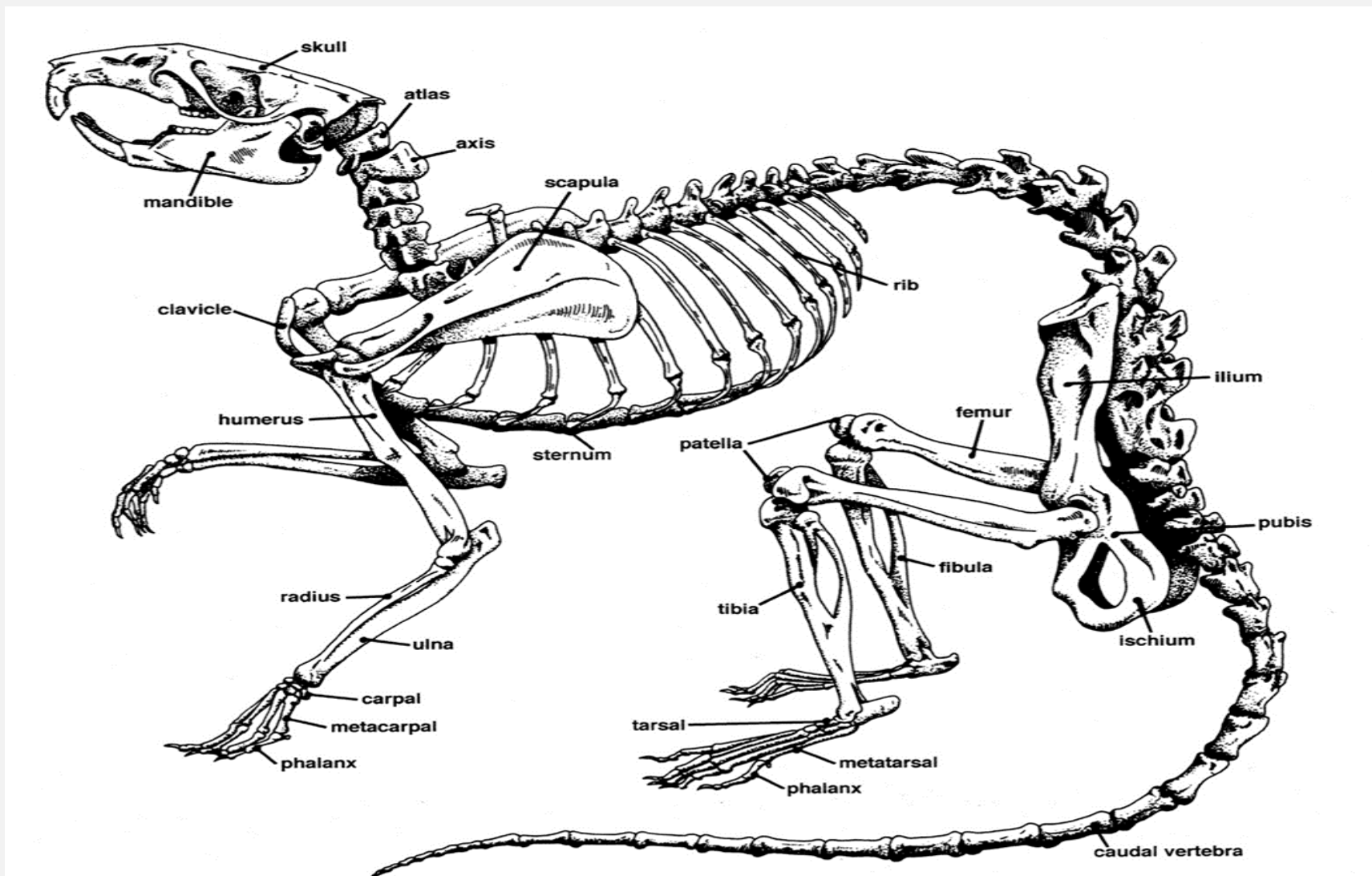
Laboratory Mouse Handbook

Skeletal Anatomy of the Mouse

Area of Concentration

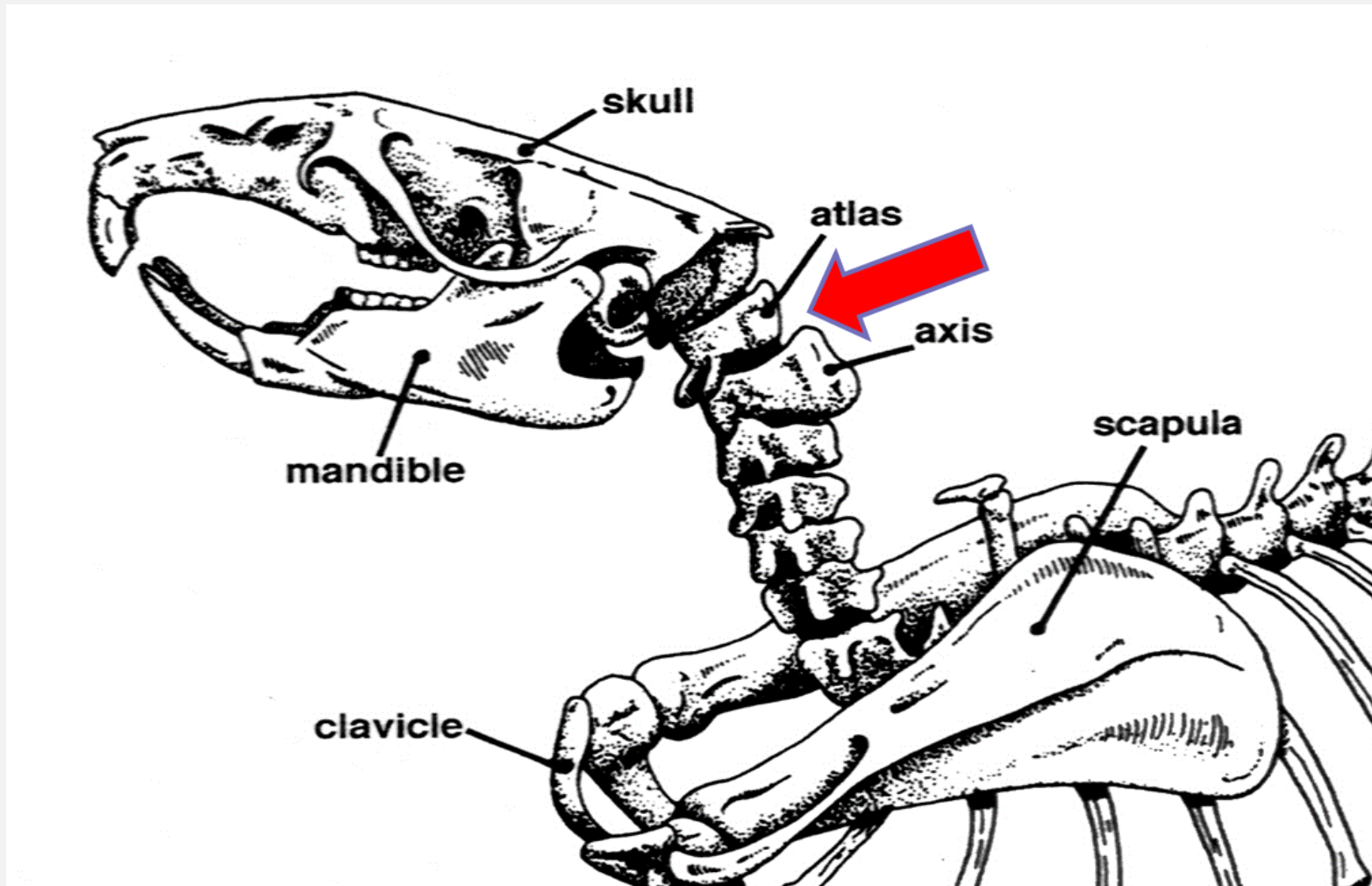


Skeletal Anatomy of the Rat



Skeletal Anatomy of the Rat

Area of Concentration



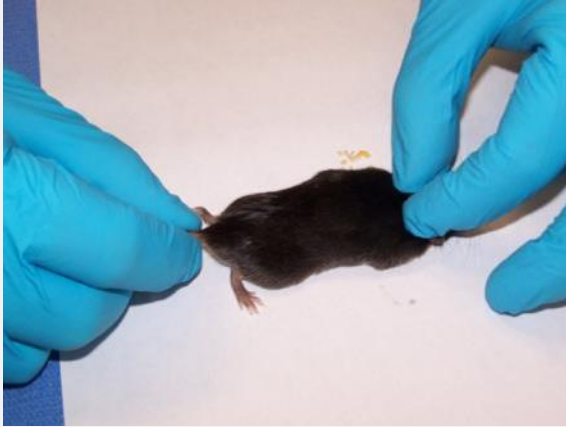
By AVMA
Guidelines
cervical
dislocation
should only be
performed on
rats 200 grams
and lighter

Cervical Dislocation Using an Instrument

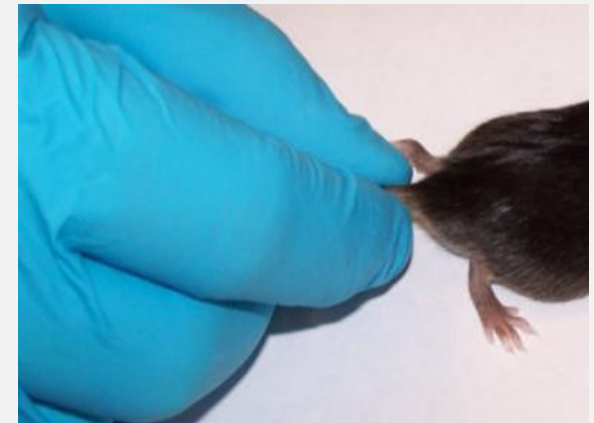
A pen, pencil or similar object may be placed directly behind the ears at the base of the skull to apply slightly forward and downward pressure to assist with the disarticulation. Grasp the base of the tail, NOT the end (as it may deglove) and with the head held securely in place, with a steady pulling motion, lift the tail slightly and disarticulate the cervical vertebrae



Cervical Dislocation Without Instruments



Grasp the mouse directly behind the ears and at the base of the tail. Do not grasp tail in the middle or at the end since it may cause it to deglove.



While keeping the head stationary, with a firm steady pulling motion lift the tail slightly and disarticulate the cervical vertebrae.

Thoracotomy



With mouse or rat on its' back, grasp a small flap of skin at the end of the sternum



With sharp scissors, cut a small hole in the skin and musculature at the diaphragm

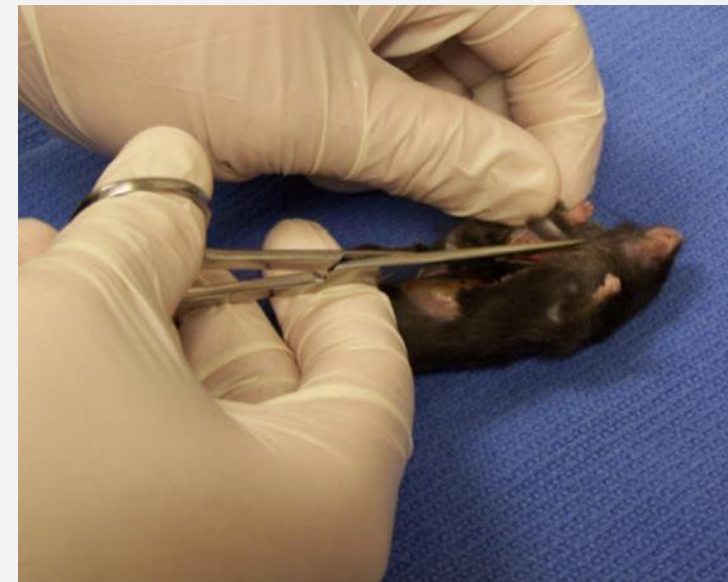


Thoracotomy



Insert one blade of scissors into the opening at the point of the sternum

Cut through the rib cage opening the thoracic cavity



Proper Handling of Rodents Post Euthanasia

IACUC requirements for proper handling of rodents after euthanasia are that **all** euthanized rodents (this includes neonates) ***Must Be***:

Placed in leak proof bags

Clearly labeled (using tape, tags, or markers) with the Principal Investigator's name OR Protocol #, your initials, and the date

If you observe someone who is not following proper euthanasia procedures, make DLAR aware of this or contact the IACUC.



Proper Handling of Rodents Post Euthanasia



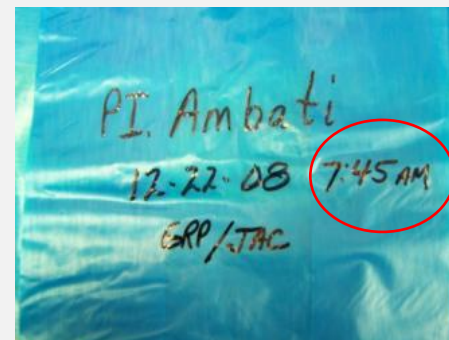
Step 1.
Properly label the body bags with PI Name or Protocol #, Date, (*time optional*), and your Initials



Step 2.
Place animals in bag and tie the opening



Step 3.
Place securely tied and labeled bag in refrigerator or cooler



Time of day is optional

Keep it Clean

Clean the chamber and surrounding area with disinfecting agent (MB-10 solution)

Place empty home cages/water bottles in designated area for dirty/used caging

Do not leave in procedure rooms



***Your mom doesn't work here so
clean up is up to you!***



Who to Call if You Need Assistance

ANIMAL CARE SUPERVISORS

NAME	OFFICE PHONE	PAGER
Kim Tomaschko (Animal Care Supervisor III)	859-323-2816	859-330-2112
Josh Murphy (Animal Care Supervisor I)	859-218-3672	859-330-1991
Peggy Hankes-Peña (Animal Care Supervisor III)	859-323-6015	859-330-0981
Erin Jones (Animal Care Supervisor II)	859-257-3562	859-330-1897
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Reference Sources & Acknowledgements

Assistant Laboratory Animal Technician Manual

(American Association for Laboratory Animal Science)

[2020 Report of the AVMA Panel on Euthanasia](#)

Policy Procedures and Guidelines:

[IACUC-103](#) (Institutional Animal Care and Use Committee, University of Kentucky)

Division of Laboratory Animal Resources Veterinarians

Research Analysts DLAR (for their assistance in graphic representation)



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