

Rodent Euthanasia Steps Using CO₂



April, 2021



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

Research

Division of Laboratory

Animal Resources



Carbon Dioxide Method

Do Not pre-fill the chamber

Use correct setting

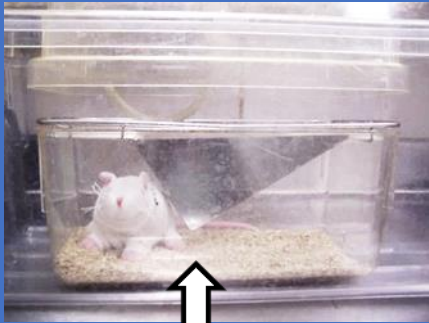
Consult wall chart for proper settings

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

Large Cage



Large Cage



Small Cage



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, pneumo-thorax, 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)

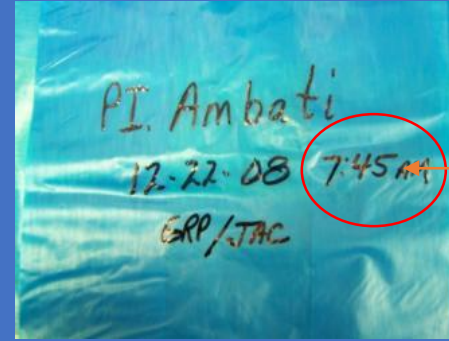
Cervical dislocation

* 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.

Proper Handling of Rodents Post Euthanasia



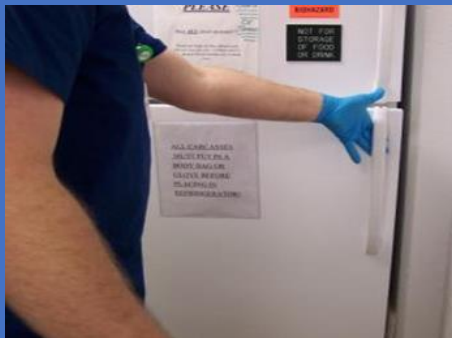
Step 1.
Properly label the body bags with PI Name or Protocol Number, Date, (*time optional*), and your Initials



Time of day is optional



Step 2.
Place animals in bag and tie the opening



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