



OFFICE OF LABORATORY ANIMAL CARE

Working Instructions

WIN Number:	108	Euthanasia Via CO2	Revision #:	0
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Author(s):	Ataki Wilson	Sign:	<i>Ataki Wilson</i>	Date:	5/2/2023
Director:	Dr. Gregory Lawson	Sign:	<i>Gregory Lawson</i>	Date:	5-2-2023

PROCEDURE

All work instructions must be demonstrated by the trainer and performed by the trainee.

1. Check equipment prior to euthanizing to ensure there is CO2 available, and equipment is functioning properly.
2. Euthanize animals in their home cage or a clean cage. Do **NOT** combine animals from different home cages.
3. Remove the cage lid. Wire bars (if present), feeders, and huts or houses should be removed.
Move rats housed in double wide cages into a clean single wide cage, if there is not a double wide cage euthanasia lid available.
4. Place the CO2 lid over the top of the cage. Make sure the inlet and outlet holes are positioned over the cage top.
5. Turn on the CO2 source (house air or tank).
6. Adjust the knob on the flow meter.
 - a) The flow is based on the volume of the cage, not necessarily the species.
 - i. Standard mouse cage: 4-6 L/min
 - ii. Standard rat cage (single wide): 6-8 L/min
 - iii. Double wide rat cage: 12-16 L/min

7. Watch for breathing to stop. CO₂ exposure times vary from animal to animal and by age. Expose animals as long as necessary.
 - a) Adult animals may take up to 5 minutes.
 - b) Neonatal rodents under 10 days old may take up to 15 minutes
 - c) Neonates 0 - 7 days could take as long as 45 minutes.
8. Do not leave animals unattended throughout the entire euthanasia process. Please take a moment to thank the animals for their service to science and education.
9. Turn off the CO₂ and remove the lid.
10. Check the animal for breathing, color and perform a toe pinch to ensure the animal is dead. No reflex or breathing should be observed. If a reflex or breathing is observed repeat the euthanasia process.
11. Perform a secondary method of euthanasia. The secondary method will be outlined in your AUP some examples are listed below. Cervical dislocation (adult mice, small juvenile rats, and birds) Decapitation (neonatal rodents, mice, rats, and birds) Bilateral thoracotomy (rodents).
12. Bag carcasses and place in the cold room, necropsy refrigerator, or freezer.
13. Clean up the work area with approved disinfectant.

REFERENCE DOCUMENTS

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REVISION HISTORY			
REVISION NUMBER	AUTHOR(S)	EFFECTIVE DATE	REVISION(S)
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