

RODENT CO2 EUTHANASIA: 2020 AVMA GUIDELINES

The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO₂, is unacceptable per [AVMA Guidelines for the Euthanasia of Animals: 2020 Edition](#). Also see [IACUC Policy #103](#) and the Office of the Attending Veterinarian (OAV) "[Guidelines for Euthanasia](#)" for additional information.

Carbon dioxide exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 30% to 70% of the chamber volume/min is recommended. One way of achieving gradual fill (displacement) needed is using a flow meter along with a pressure-reducing regulator.

Using this gradual fill (displacement) method, CO₂ flow should be maintained for at least 1 minute after respiratory arrest. If animals need to be combined, they should be of the same species and, if needed, restrained so that they will not hurt themselves or others. Immature animals must be exposed to high concentrations of CO₂ for an extended period of time to ensure death. In all cases a secondary method to ensure death is necessary (see IACUC#103)

It is preferable that animals are euthanized in their home cage to minimize stress. If this is not possible, the euthanasia chamber must be emptied and cleaned between uses. 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. The CO₂ flow should be set to displace 30-70% of the chamber volume per minute:

Flow Rates for 30-70% Volume Displacement per Minute

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

DLAR has flow meters and [instructions for flow rate](#) on all euthanasia stations to comply with the gradual fill method (See Page Below). It is recommended you contact your compressed gas supplier for options of regulator/flow meter. Depending on compatibility of your euthanasia equipment with DLARs, DLAR may be able assist investigators needing to obtain and/or install flow meters for use in their CO₂ euthanasia stations.

For Questions regarding CO₂ Flow meters/Regulators or Installation contact DLAR Surgery Tech [Wade Washington](#) at 323-6027. A Service Charge will be assessed for installation at the Vet Tech Time rate. [For current rates visit the Per Diems page on the DLAR on the DLAR website.](#)

RODENT CO2 EUTHANASIA: 2020 AVMA GUIDELINES

The following statement is recommended for inclusion in an Animal Use Protocol for any species where CO2 euthanasia is to be used:

“Animals will be euthanized using a 30-70% per minute displacement of chamber air with compressed CO2. Following unconsciousness/death the animals will be subject to cervical dislocation/decapitation/thoracotomy as a secondary means to ensure death.”

Reference:

1. AVMA Guidelines for the Euthanasia of Animals:2020, pp28-31
2. IACUC Policy #103
3. OAV “Guidelines for Euthanasia”
4. DLAR Rodent Euthanasia Steps Using CO₂

DLAR CO₂ Regulators/ Flow Meters



WTF CO2F-191M4 CO2 Flow Meter 0-12LPM ¼ NPT M



WTF CO2R-320 CO2 Regulator 50PSI Preset, CGA
CGA 320 ¼ NPT F Outlet



3058301-01-M1D CO2 Regulator, CGA 320 with
0-12 LPM Flowmeter

****For current pricing contact American Welding & Gas****

Thoroughbred Ind Cylinder Exchange
1575 Winchester Road, Suite 160 Lexington, KY 40505
Office (859) 737-8142