CO_2 Euthanasia – revised guidelines

The following additions have been made to the UCAR guidelines for the Use of CO_2 for Small Animal Euthanasia:

1. All euthanasia chambers must be transparent so that all animals can be observed during the process.
2. A CO_2 euthanasia SOP must be developed and posted for each location where CO_2 euthanasia is performed. The SOP must meet UCAR guidelines.
3. Flow to the euthanasia chamber **must be calibrated** to displace at least 20% - 70% of the chamber volume per minute in your set up. Your settings will vary for different sized chambers. The recommended way to insure that the system is achieving appropriate flow is by using a flow meter. Alternately, if you are using a 2-stage regulator, flow should be fairly reproducible for a given pressure at the second stage. You can use a flow meter to determine the appropriate pressure range that allows your regulator to deliver the appropriate volume of CO_2. If you wish to calibrate your system in another way, please state clearly how it will be done, and provide all calculations.

Use of CO_2 for Euthanasia

The Office of Laboratory Animal Welfare (OLAW) provided clarification for current requirements for using CO_2 as a euthanasia agent for small laboratory animals. Compressed CO_2 gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely.

In the Vivarium proper, you may use a CO_2 euthanasia station, which consists of a gas tank and a regulator which is connected to a lid that will fit on a mouse or a rat home cage. Directions and settings are provided in each room.

When performing CO_2 euthanasia in a laboratory follow specific guidelines.

The acceptability of using CO2 as a euthanasia agent is predicated on the following:

- High concentrations of CO2 may be distressful to some species. Accordingly, pre-filling the chamber is recommended only under circumstances in which such use has not been shown to cause distress. This should not be done for rats or mice.
- All euthanasia chambers must be transparent so that all animals can be observed during the process.
- Chambers must not be overcrowded. In this regard, it is important to also consider that mixing unfamiliar or incompatible animals in the same container may be distressful.
- A CO_2 euthanasia SOP must be developed and posted for each location where CO_2 euthanasia is performed. The SOP must meet UCAR guidelines.

- **Compressed CO2 in cylinders** is the only AVMA Panel-recommended source of CO2 for euthanasia or sedation purposes. Dry ice is not an approved source of carbon dioxide.
- Flow to the euthanasia chamber **must be calibrated** to displace at least 20% - 70% of the chamber volume per minute in your set up. Your settings will vary for different sized chambers. The recommended way to insure that the system is achieving appropriate flow is by using a flow meter. Alternately, if you are using a 2-stage regulator, flow should be fairly reproducible for a given pressure at the second stage. You can use a flow meter to determine the appropriate pressure range that allows your regulator to deliver the appropriate volume of CO_2. If you wish to calibrate your system in another way, please state clearly how it will be done, and provide all calculations.
- Death must be verified after euthanasia and prior to disposal. Unintended recovery must be obviated by the use of appropriate CO2 concentrations and exposure times or by other means. OLAW notes that thoracotomy after apparent death from CO2 is one way to ensure the irreversibility of the procedure.
- UCAR requires that a secondary physical method be performed to ensure that the animals are dead. The recommended secondary physical methods are: decapitation, perfusion of a histological fixative via the major blood vessels, pneumothorax by opening the thorax, complete severing of the spine just below the base of the skull using a dorsal approach and cervical dislocation for animals under 200g.

It is important that you understand that unintended recovery of animals after apparent death from CO2 (e.g., in a necropsy cooler, morgue cooler) is a serious noncompliance issue. It will be reported to OLAW and could result in loss of privileges. For more information refer to http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html.

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