**IACUC Training Exercise #2 2014**

The following exercise may be useful in stimulating discussion regarding compliance with PHS Policy and VA Handbook 1200.07. To facilitate discussion, page 1 of the exercise may be distributed to the IACUC members prior to a meeting. After a few minutes of discussion about the exercise during the meeting, the remaining pages of the exercise may be distributed to provide ideas for the committee’s consideration.

Recent discussions between the CVMO and the Office of Laboratory Animal Welfare (OLAW) regarding carbon dioxide (CO2) euthanasia brought to our attention that some programs have not yet fully adopted the recommendations outlined in the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.*  The CVMO’s office reminds all personnel involved in the animal care and use program that each CO2 euthanasia system no matter where it is located (in the animal facility or in an investigator laboratory), must have a flow regulator, and the flow rate must be calculated for the volume of the specific euthanasia chamber in use.

1. **Practices that comply with current AVMA guidance on CO2  euthanasia1:**
* A gradual fill method is less likely to cause pain prior to unconsciousness.
* A displacement rate from 10% to 30% of the chamber volume/min is recommended by the AVMA Euthanasia Guidelines published in 2013.  OLAW interprets this recommendation very strictly (see: <http://grants.nih.gov/grants/olaw/130919_AVMA_slides.pdf>).
* The CO2 flow should be continued for at least 1 minute after respiratory arrest, if the gradual displacement methods are used.
* Only use a precisely regulated and purified form of CO2 without contaminants or adulterants (i.e. commer­cially supplied cylinder or tank).
* “An appropriate pres­sure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size con­tainer being utilized is absolutely necessary.”1

**B.  To calculate flow rate:**

* Determine volume of euthanasia chamber. Length x width x height of euthanasia chamber in inches = chamber volume in cubic inches;
* 1 liter = 61.02 cubic inches, so divide the total cubic inches by 61.02 cubic inches/liter to get the volume of the chamber in liters.
* Calculate flow rate needed for 10-30% displacement of chamber volume per minute.  Multiply chamber volume in liters by displacement rate (0.1 to 0.3) to get the needed regulator flow rate.

 Practice problem:An IACUC approves a 30% displacement rate for rodent carbon dioxide euthanasia.  The euthanasia chamber is in the shape of a cube, with each side measuring 20 inches.  The flow regulator on the carbon dioxide tank is in liters per minute.  To what value should the flow meter be set for this chamber to provide 30% displacement per minute by carbon dioxide gas?

Practice problem answer:

Volume of chamber= 20.0 in x 20.0 in x 20.0 in= 8,000 cubic inches; 8,000 cubic inches/ 61.02 cubic inches/liter= 131 liters

131 liters x 0.3 displacement/minute= 39.3 liters/minute

Answer- the flow regulator should be set to about 39 liters per minute.

*Note: The CVMO’s office will be happy to assist with double-checking flow calculations.*

**C.  When performing CO2 euthanasia, several factors may influence not only the time to death but also the level of distress experienced by the animals (see below).**

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| **Factors** | **Rationale** |
| Species | Rabbits tend to have a prolonged survival times when exposed to CO2 in comparison to rodents.1 |
| Age | Neonatal rat and mouse pups may take nearly an hour of exposure to CO2 to ensure death.1    |
| CO2 entry point | Filling the chamber from the top of the chamber mixes the CO2 more effectively than filling from the bottom; bottom filling may result in localized high CO2 levels or a layering effect.  Animals will try to avoid CO2 by raising their heads, jumping or climbing. 1  |
| Overcrowding | Crowding may result in inadequate mixing of the CO2 with the air in the cage, so that there may be pockets where the animals are over- or under-exposed.  Sufficient space and free flow of CO2 to each animal will occur if the animals are able to assume normal postures and turn around in the euthanasia chamber.2 |
| Displacement rate | “Use of 100% CO2 at a flow rate of 20% of the chamber volume per minute has been shown to produce loss of consciousness without evidence of pain, but not without evidence of dyspnoea. Reduced flow rates can be increased once animals have lost consciousness.”3  A 10% per minute displacement of CO2 appears to result in lower heart rates and sedation but significantly lengthens the time to unconsciousness (156 seconds) and death (14.17 + 3.66 minutes), and does not necessarily mean that death was stress-free.4    “Prolonged exposure to low concentrations of CO2 (6% to 10%) has been found to increase corticosterone in rats…”1, whichsuggests distress.   |

**D.  Sources:**

**1** "Guidelines for the Euthanasia of Animals: 2013 Edition." AVMA, 27 Feb. 2013. Web. 15 May 2013. <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

**2** "UGA IACUC Policy on Rodent Euthanasia Using C02." Office of the Vice President   for Research, 18 May 2006. Web. 15 May 2013. <http://www.ovpr.uga.edu/docs/policies/compliance/C02-Euthanasia.pdf>

**3** Hawkins P., Playle L., Coledge H., Leach M., Banzett R., Coenen A., Cooper J., Danneman P.,   Flecknell P., Kirkden R., Niel L., and Raj M. "Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals." UK, Tyne. 2006. 1-17. 9 Aug. 2006. Web. 15 May 2013. <http://www.nc3rs.org.uk/downloaddoc.asp?id=416&page=292&skin=0>

**4** Burkholder, Tanya H., Lee Niel, James L. Weed, Laruen R. Brinster, John D. Bacher, and Foltz J. Foltz. "Comparison of Carbon Dioxide and Argon Euthanasia: Effects on Behavior, Heart Rate, and Respiratory Lesions in Rats." *Journal of American Association for Laboratory Animal Science* July 49.4 (2010): 448-53. July 2010. Web. 15 May 2013. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2919185/>

**5** Instructions: Carbon Dioxide Euthanasia.” Boston University Research Compliance Animal Care, 2013. Web. 1 Jul 2014. <http://www.bu.edu/animalcare/lasc-bumc/guidelines/3-level-page/>