The following scenario may be useful in stimulating discussion about implementing the new AVMA recommendations on carbon dioxide euthanasia of rodents. To facilitate discussion, page 1 of the scenario may be distributed prior to the IACUC meeting. After a few minutes of discussion during the IACUC meeting, the remaining pages may be distributed to provide ideas for the committee’s consideration.

Most people at the Hometown VAMC thought Dr. Matthew Maverick’s surname fit him perfectly. His zeal for being independent and often unconventional had helped him to become a well-funded diabetes investigator but it also occasionally got him into trouble. At the moment, he was in a bind because there was no one in the lab to carry out his research. His lab manager, Bonnie, was away for three weeks on a vacation in Hawaii, his post-doc had left to start his first faculty appointment, and his new graduate student wasn’t starting for another month. He had little choice but to do the experiments himself; thankfully, Bonnie had left a complete list of everything that needed to be done while she was away. Today, he was supposed to euthanize a group of rats and collect tissues. Bonnie always used the carbon dioxide (CO2) euthanasia set-up in the animal facility to euthanize the rats but Dr. Maverick discovered there were many other people waiting to do the same thing when he walked in. His time was entirely too valuable for him to stand in line, so he immediately decided he would rig up his own CO2 chamber in his lab. He checked the CO2 incubator to make sure no one had started any cell cultures. The incubator was empty, so he disconnected the CO2 supply tubing from the incubator and discovered that it fit nicely through the grommet for the automated watering lixit located near the bottom of an empty rat cage. Dr. Maverick pushed the tubing through the grommet opening so it would not become dislodged once CO2 began to flow into the cage. Maverick had brought three cages of rats up from the animal facility. Each cage contained three rats; he decided it would be quickest and easiest to euthanize all the rats at once, so he put all nine into the cage that he had rigged to receive the CO2. Then, Dr. Maverick turned the CO2 valve wide open. To his surprise, the rats immediately started jumping about the cage, salivating, and frantically pawing their faces. As luck would have it, Dr. Diaz, the Attending Veterinarian, walked into Dr. Maverick’s laboratory just in time to see the frantic rats suddenly collapse. Dr. Maverick was red-faced and Dr. Diaz was dumbstruck. After she recovered, she helped Dr. Maverick (appropriately) finish euthanizing the rats and collect the tissues he needed. Then, Dr. Diaz calmly said, “I think we need to review the correct way to use CO2 to euthanize rodents.”

**What was incorrect about the way Dr. Maverick euthanized the rats? What advice about CO2 euthanasia do you think Dr. Diaz gave to Dr. Maverick?**

**What mistakes did Dr. Maverick make?**

Dr. Maverick made several mistakes in setting up the CO2 euthanasia chamber in his lab. The first mistake was to use the grommet near the bottom of the cage for the CO2 inlet so filling occurred from the floor of the cage. The second was to put nine rats into a cage intended to hold three rats. Finally, he had no idea how much CO2 he was dumping into cage. The table below reviews three important factors to be considered when performing CO2 euthanasia.

|  |  |
| --- | --- |
| **Factors** | **Rationale** |
| 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 | Exposure to 100% CO2 causes bradycardia within less than 3 seconds and irritates the mucosa of the eyes, nose, and throat.3 Animals are unconscious in approximately 15 seconds but experience at least 10 to 15 seconds of pain and distress.3 “The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO2, is unacceptable.”1   “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.  |

**What did Dr. Diaz recommend?**

Dr. Diaz referred Dr. Maverick to the recently published *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* and reviewed the recommendations for CO2 euthanasia with him. The main take-home points were as follows:

* 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.
* 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

Dr. Diaz reminded Dr. Maverick that euthanizing animals in their home cage is considered to be less stressful than transferring them to an unfamiliar cage. Species and age also influence CO2 exposure time; for example, neonatal rat and mouse pups may take nearly an hour of exposure to CO2 to ensure death.1 Rabbits are also know to have prolonged survival times when exposed to CO2.1 Consequently, animals that are to be euthanatized together should be of the same species and of similar age. Dr. Maverick agreed that the recommendations made sense but he still wasn’t clear about how to calculate the displacement rate. Dr. Diaz explained that if the rat cage dimensions were 47 cm/18.5 in (L) X 28 cm/11 in (W) X 22.9 cm/9 in (H), and the desired displacement rate is 20%, the flow in liters per minute could be calculated using either of the formulas below:

 **L (cm) X W (cm) X H (cm) x 1/1000 = volume of chamber in liters**

47 X 28 X 22.9 X 1/1000 = 30.1

**Volume in liters** $×$ **% chamber displacement/min = flow rate in liters/min (l/min)**

30.1 X 0.20 = 6 l/min

OR

 **L (in) X W (in) X H (in) X % chamber displacement/min = flow rate in cubic inches/min (in3/min)**

18.5 X 11 X 9 X 0.20 = 366.3

**Flow rate in in3/min ÷ \*61.024 in3 = flow rate in liters/min (l/min)**

366.3 ÷ 61.024 = 6 l/min

\* 61.024 cubic inches (in3)) = 1 liter 5

Dr. Diaz and Dr. Maverick agreed that it was in his best interest to use the CO2 euthanasia set-up in the animal facility, follow the posted standard operating procedure (SOP) for CO2 using the gradual displacement method, and that she would supervise the euthanasia of the group of rats he planned to euthanize tomorrow.

The recommendations of the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* should be adhered to unless a deviation is justified for scientific or medical reasons.6 The *Guide*  states “In evaluating the appropriateness of methods, some of the criteria that should be considered are ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; irre­versibility; time required to induce unconsciousness; appropriateness for the species and age of the animal; compatibility with research objectives; and the safety of and emotional effect on personnel.”6 Each station should develop a CO2 euthanasia SOP based on testing of the equipment used in the local facilities. If the data collected by the station indicate that a displacement rate other than that recommended by the AVMA is more appropriate as defined above, then the IACUC may approve it as a specifically described exception based on a scientific or medical reason.

**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** "Liter." *The Free Dictionary*. Farlex, 2013. Web. 15 May 2013. <http://encyclopedia2.thefreedictionary.com/Lite>r.

**6** *Guide for the Care and Use of Laboratory Animals*. National Academy of Sciences, 2011. Web. 15 May 2013. [*http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf*](http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf).