CCAC REVISED GUIDANCE ON EUTHANASIA USING CARBON DIOXIDE

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This guidance note replaces the guidance concerning carbon dioxide in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010). The *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) was based on the International Council for Laboratory Animal Science principles for euthanasia in *Harmonization of animal care and use guidance* (Demers et al., 2006), which in turn was based on the American Veterinary Medical Association (AVMA) *Guidelines on Euthanasia* (AVMA, 2007); and *European Recommendations for Euthanasia of Experimental Animals: Part 1* (Close et al., 1996) and *European Recommendations for Euthanasia of Experimental Animals: Part 2* (Close et al., 1997), adapted for the Canadian context.

Following the revision of the AVMA guidelines on euthanasia and the publication of the 2020 edition (AVMA, 2020), the CCAC has revisited its guidance on the use of carbon dioxide to kill rodents. In the view of the CCAC, the most significant change to the AVMA guidance is the increase in the upper limit of the carbon dioxide flow rate to 70% of the euthanasia chamber volume per minute. In response to this, and based on the most recent scientific literature and expert opinion, the CCAC has changed its guidance on carbon dioxide flow rate to 30-40% of the chamber volume per minute (previously 20-30%), with animal care committees having the discretion to allow higher flow rates if deemed appropriate. Maintaining the concentration of carbon dioxide in the euthanasia chamber below 40% before rodents become unconscious is key to ensuring that rodents do not experience pain. The flow rate recommended by these revised guidelines (i.e., 30-40%) has been set to ensure that rodents experience only minimal pain and distress prior to death, recognizing that institutions may not have the ability to monitor the actual carbon dioxide concentration within the euthanasia chamber. Where institutions can show that their particular euthanasia set-up does not permit the concentration of carbon dioxide within the chamber to rise above 40% at higher flow rates (prior to the animals becoming unconscious), these higher flow rates may be used, if approved by the animal care committee.

The *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) will be revised within the next few years, and the matter of carbon dioxide will be revisited at that time. In the interim, CCAC expectations for institutions using carbon dioxide are listed below.

SUMMARY OF REVISED GUIDANCE

1. Carbon dioxide should not be used where other methods are practical for the experiment and the species.
2. Immersion of animals into chambers pre-filled with 100% carbon dioxide is unacceptable.
3. The use of a gradual-fill method is conditionally acceptable for euthanasia using carbon dioxide for some species, as noted in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010).
4. As a conditionally acceptable method, any euthanasia using gradual-fill carbon dioxide on non-anesthetized rodents must be justified to and approved by an animal care committee before it is permitted to take place.
5. If carbon dioxide is required for non-anesthetized rodents, a gradual-fill rate of less than 40% and greater than 30% of the chamber volume per minute should be used.
6. As part of the approval process, the animal care committee should determine the acceptability of displacement rates greater than 40% of the chamber volume per minute, based on the most current research and on local considerations.
7. Where isoflurane is used to anesthetize the animals prior to euthanasia with carbon dioxide, care should be taken to allow sufficient time to wash in carbon dioxide prior to the wash-out of isoflurane, so that the animals do not recover during the switch.

REVISED GUIDANCE, IN DETAIL

1. Carbon dioxide should not be used where other methods are practical for the experiment and the species.

The use of carbon dioxide is not an acceptable method to kill animals used in science, as it does not fulfill two of the key criteria for "euthanasia":

- euthanasia should result in rapid loss of consciousness, followed by respiratory and cardiac arrest and ultimate loss of all brain function; and
- euthanasia should aim to minimize any pain and distress experienced by the animal prior to loss of consciousness.

Wherever possible, an acceptable method (as listed in the *CCAC guidelines on: euthanasia of animals used in science* [CCAC, 2010]) should be selected. There are, however, instances when it is not practical to use acceptable methods such as an overdose of an inhalant anesthetic or injection of pentobarbital (e.g., the need to kill significant numbers of surplus animals).

2. Immersion of animals into chambers pre-filled with 100% carbon dioxide is unacceptable.

There is no ideal way of killing animals with carbon dioxide. However, immersion of animals into chambers pre-filled with 100% carbon dioxide is inhumane, due to the extreme pain and distress caused by the formation of carbonic acid on the mucous membranes of the airways and the intense panic reaction triggered by carbon dioxide receptors that signal "air hunger" and result in gasping.

3. The use of a gradual-fill method of carbon dioxide is a conditionally acceptable method for killing certain species, as noted in the *CCAC guidelines on: euthanasia of animals used in science*.

Conditionally acceptable methods for killing experimental animals may be used in certain circumstances when there is:

- scientific justification;
- review and approval by an animal care committee; and
- assurance that trained personnel to conduct the euthanasia are available.

"These are not considered 'acceptable methods […] because there is greater potential for operator error or safety hazards, they might not consistently produce humane death, or they are not well documented in the scientific literature. When conditionally acceptable methods that have been approved by the animal care committee are used, the conditions of use and training of the personnel involved should be clearly stated in the protocol" (CCAC, 2010). The use of carbon dioxide falls into this category.

4. As a conditionally acceptable method, any euthanasia using gradual-fill carbon dioxide on non-anesthetized rodents must be justified to and approved by an animal care committee before it is permitted to take place.

Animal care committees should assure themselves that there is no acceptable alternative before approving the use of carbon dioxide. Each institution has its own particular needs and equipment set-up. Animal care committees may choose to approve detailed species-specific standard operating procedures developed to ensure that when carbon dioxide needs to be used, the method employs the most humane conditions. Detailed standard operating procedures for appropriate use of carbon dioxide should reflect the latest guidelines (i.e., use of compressed carbon dioxide (not dry ice) with a flow meter so that carbon dioxide can be delivered to the chamber at an appropriate rate, running sufficiently long to ensure death; use of a secondary method to ensure death; and flushing the chamber in between cages/groups).

5. If carbon dioxide is required for non-anesthetized rodents, a gradual-fill rate of less than 40% and greater than 30% of the chamber volume per minute should be used.

Based on the most recent scientific literature, the CCAC has changed its guidance on the flow rate to 30-40% of the chamber volume per minute. There is evidence that breathing carbon dioxide at any concentration higher than approximately 15% causes anxiety and distress due to dyspnea ("air hunger"), and breathing carbon dioxide at a concentration higher than approximately 40% causes pain due to the formation of carbonic acid on the mucous membranes of the eyes, nose, and respiratory tract.

When carbon dioxide is administered into a chamber at a low flow rate, the time to unconsciousness is long, but the concentration in the chamber is low at the time of loss of consciousness. When carbon dioxide is administered at a high flow rate, the time to unconsciousness is shorter, but the concentration in the chamber is higher at the time of loss of consciousness. Flow rates of 30-40% of the chamber volume per minute shorten the time to unconsciousness (and thus reduce the time animals experience anxiety and distress due to

dyspnea), while ensuring most animals are unconscious before they experience pain. At flow rates higher than 40%, the time to unconsciousness is even shorter, but animals will experience pain prior to losing consciousness.

6. **As part of the approval process, the animal care committee should determine the acceptability of displacement rates greater than 40% of the chamber volume per minute, based on the most current research and on local considerations.**

Each institution will have different equipment set-ups. Accordingly, animal care committees may choose to approve the use of flow rates greater than those recommended in the current guidance. In order to do so, they must be convinced that the higher flow rates will not cause undue pain for the animals (potentially by increasing the chamber carbon dioxide concentration above 40% too quickly). This may require monitoring the concentration of carbon dioxide within the euthanasia chamber.

7. **Where isoflurane is used to anesthetize the animals prior to euthanasia with carbon dioxide, care should be taken to allow sufficient time to wash in carbon dioxide prior to the wash-out of isoflurane, so that the animals do not recover during the switch.**

In general, overdose of an inhalation anesthetic agent is an effective method of euthanasia for many species and is recognized as an acceptable procedure, particularly at the end of a non-recovery procedure when the anesthetic level can be increased or a secondary method can be used to ensure death. Isoflurane can be used to anesthetize animals prior to killing them with carbon dioxide. This is particularly appropriate for animals that have not been exposed to isoflurane previously, as a repeat exposure to isoflurane appears to be aversive to rodents (although not as aversive as exposure to carbon dioxide). However, if the switch to carbon dioxide is not properly managed, there is the risk that the animals may recover from the anesthetic and regain consciousness while the level of carbon dioxide is rising, resulting in air hunger.

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