Implementation of the *CCAC guidelines on: euthanasia of animals used in science*

The *CCAC guidelines on: euthanasia of animals used in science* (2010) was developed by the CCAC ad hoc subcommittee on euthanasia[1] and supersedes information contained in Chapter XII—Euthanasia in the *Guide to the Care and Use of Experimental Animals*, vol. 1, 2nd ed. (1993)[2]. Comments received from three external reviews of this guidelines document[3] and a presentation at a scientific meeting[4] contributed substantially to the development of a comprehensive set of guidelines with supporting context to facilitate the implementation of best practices.

These guidelines apply to animals used by institutions for scientific purposes, with a particular focus on animals used in a laboratory setting. They aim to provide updated information for investigators, animal care committees (ACCs), facility managers, veterinarians and animal care staff that will assist in establishing and reviewing procedures for euthanasia of animals in their care. The refinement of animal care and use is a continuous process. In this respect, the guidelines provide a framework for the implementation of best practices.

Euthanasia means a gentle death, and in the context of animals used in science, refers to 'humane killing' or doing what is humanly possible to minimize pain and distress, given the circumstances, including the research goals, under which euthanasia is performed. The most important criteria for acceptance of a method of euthanasia is that it have a rapid initial depressive action on the central nervous system to ensure immediate insensitivity to pain, and that steps are taken to minimize distress for the animal prior to the procedure.

Decisions regarding the best method of euthanasia should take into account the competence of the personnel involved, the age and condition of the animal, and the experimental protocol. Investigators should consult with the veterinarian on staff and any other pertinent resources to develop the best approach for the specific circumstances. Euthanasia of any experimental animal should never be undertaken by anyone who is not fully competent in the procedure and must always be done using the appropriate equipment required to perform the procedure humanely. An important consideration in the choice of method of euthanasia is the impact on the goals of the research. An addendum to this guidelines document is under development, which will provide information on the possible impacts of various methods of euthanasia on research results.

The *CCAC guidelines on: euthanasia of animals used in science* is based on recommendations made by the International Council for Laboratory Animal Science (ICLAS) Working Group on Harmonization and the two international reference documents on euthanasia recommended by ICLAS: the American Veterinary Medical Association (AVMA) *Guidelines on Euthanasia* (2007) and the Working Party Report to the European Commission, *Recommendations for euthanasia of experimental animals*, Part 1(1996) and Part 2 (1997). Through the work of the subcommittee and feedback from reviewers, it was realized that an explanation was needed for each of the guiding principles, and some of the principles needed rewording to better fit the Canadian context in which this guidelines document would be used. Additionally, there was a need to clarify some information presented in the AVMA and European documents, and to include recent scientific evidence for particular methods of euthanasia.

This guidelines document provides a discussion of the general guiding principles on euthanasia agreed upon by ICLAS and an overview of acceptable methods of euthanasia for common species used in science, as well as information on other methods of euthanasia. In this document, "acceptable methods" are methods that are simple to perform and consistently produce death with minimal pain and distress when used on conscious or sedated animals. Other methods of euthanasia may be

acceptable (i.e. equivalent to those methods specifically listed as acceptable) when used on anesthetized or unconscious animals. "Conditionally acceptable methods" are those that may be acceptable for use in certain circumstances where there is scientific justification and following review and approval by an ACC and assurance that trained personnel are available. These are not listed as "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.

Two methods that are listed as conditionally acceptable raised particular concern by reviewers of draft versions of this document: carbon dioxide (CO_2) (Section 5.1) and T-61 (Section 5.3). One of the challenges in developing guidelines on euthanasia was the lack of scientific evidence and suitable alternatives in some areas, such as the use of CO_2. As a result the guidelines focus on best practices while recognizing that in a particular situation it may be determined that a different approach is warranted. For CO_2, the guidelines state that there is evidence to show CO_2 is aversive to rats and mice, and therefore it is not a recommended method. However, the document also recognizes that CO_2 is a commonly used method of euthanasia for rodents, particularly when large numbers are involved, and states that if CO_2 is used, the animals should ideally be anesthetized first. It is further acknowledged that there may be situations where this is not possible, and recommendations are provided for the least aversive delivery of CO_2 (i.e. a gradual fill rate of less than 30% and more than 20% of the chamber volume/minute).

While this guidelines document states that T-61 is not a recommended method for any species, the use of T-61 is listed as conditionally acceptable for some species. Earlier mention of T-61 in the *Guide to the Care and Use of Experimental animals* indicated problems associated with improper administration, and this has been further explained in the current guidelines document. The *guidelines on: euthanasia of animals used in science* cautions ACCs to review its application and be aware of its mechanism of action when reviewing protocols requesting its use. The document also states that where possible, a sedative should be administered prior to the use of T-61 to protect the animal from any adverse effects that may be associated with the accidental failure of the procedure.

The *CCAC guidelines on: euthanasia of animals used in science* will begin to be fully implemented by the CCAC Assessment Program in January 2012, after an introductory period of one year. In the meantime, any questions concerning further clarification of the guidelines should be directed to the CCAC Guidelines Program.

[1] CCAC ad hoc subcommittee on euthanasia: Drs. Ronald Charbonneau, Centre Hospitalier de l'Université Laval; Lee Niel, University of Toronto; Ernest Olfert, University of Saskatchewan; Marina von Keyserlingk, University of British Columbia; and Gilly Griffin, CCAC. Assistance was also provided by Dr. Andrew Fletch, McMaster University, and Ms. Joanna Makowska, University of British Columbia.

[2] In the interim, the CCAC Assessment Program used the *2000 Report of the AVMA Panel on Euthanasia*. However, the Assessment Program will now be using the *CCAC guidelines on: euthanasia of animals used in science* (2010) as the main guidance document in this area.

[3] Reviews: peer review from June 5 to July 21, 2006; widespread review from July 26 to September 29, 2007; and final review from June 10 to July 17, 2009. These reviews resulted in 32, 33 and 35 sets of comments, respectively, and included reviewers from Canadian and international institutions.

[4] The draft guidelines were presented at the Canadian Association for Laboratory Animal Science (CALAS) Symposium in Québec City on April 27, 2010.