

## **Frequently Asked Questions for the *CCAC guidelines on: euthanasia of animals used in science***

Frequently asked questions (FAQs) have been developed to assist investigators and members of animal care committees (ACCs) in the implementation of the *CCAC guidelines on: euthanasia of animal used in science*. FAQs provide a generic response to some of the comments and questions received by the CCAC during the three draft reviews of this guidelines document.

If you do not find the answer to your question here, please contact the CCAC and we will be pleased to provide assistance. The FAQs will be updated regularly and will continue to reflect questions asked by ACCs and investigators in implementation of the *CCAC guidelines on: euthanasia of animal used in science*.

### **1. What is the difference between an acceptable method and a conditionally acceptable method?**

Acceptable methods are those that are simple to perform and consistently produce death with minimal pain and distress when used on conscious or sedated animals. Conditionally acceptable methods are methods 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 to carry out the procedure. 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. Some methods that are not listed as acceptable may be considered by the ACC to be acceptable (i.e. equivalent to those methods specifically listed as acceptable) when used on anesthetized or unconscious animals.

Regardless of whether a proposed method of euthanasia is listed in the *CCAC guidelines on: euthanasia of animal used in science* as acceptable or conditionally acceptable, or is not mentioned in this document, it must always be submitted for review and approval by an ACC.

### **2. What about methods not mentioned in the guidelines as acceptable or conditionally acceptable – are they considered unacceptable?**

For practical reasons, not all possible methods of euthanasia and situations have been described in the *CCAC guidelines on: euthanasia of animal used in science*. Use of any methods that have not been specifically mentioned or have not been mentioned with respect to a particular species should be discussed with a veterinarian knowledgeable about the species in question.

### **3. In some cases, the CCAC guidelines differ from one or both of the major reference documents. How is this to be interpreted?**

While the *CCAC guidelines on: euthanasia of animal used in science* draws from the two international reference documents on euthanasia recommended by the International Council for Laboratory Animal Science (ICLAS), namely the American Veterinary Medical Association (AVMA) *Guidelines on Euthanasia* (2007) and the *Recommendations for euthanasia of experimental animals* Part 1(1996) and Part 2 (1997), there are some differences. In such cases,

the CCAC guidelines are to be considered the standard for euthanasia of animals used in science in Canada.

For example, recent evidence in the scientific literature indicates that carbon dioxide is aversive to rodents (see Section 5.1 Carbon dioxide in the *CCAC guidelines on: euthanasia of animal used in science*); however, the AVMA guidelines and the European recommendations both note that carbon dioxide is an acceptable method of euthanasia. The CCAC subcommittee on euthanasia determined that current best practice is to list carbon dioxide as conditionally acceptable and state that it should not be used where other methods are practical for the experiment and the species.

As another example, while the CCAC guidelines list clove oil as an acceptable method of euthanasia for fish, the AVMA Guidelines on Euthanasia states that the use of clove oil is not acceptable because there have not been adequate and appropriate clinical trials to evaluate the effects. In reviewing the literature, the CCAC subcommittee on euthanasia determined that there was currently sufficient evidence available to support the use of clove oil as an acceptable method of euthanasia for fish.

#### **4. The guidelines state that euthanasia of any experimental animal should never be undertaken by anyone who is not fully competent in the procedure. How is competency determined?**

Institutions are responsible for determining the actual training requirements and the competency of personnel, working with those responsible for training in practice and with the institutional ACCs. In particular, ACCs should ensure that all personnel listed on an animal use protocol have received the appropriate training before the protocol is undertaken.

#### **5. How do the guidelines apply in the case of an emergency?**

The guidelines note that all proposed methods for euthanasia of animals, including emergency euthanasia, must be submitted for review and approval by an ACC. However, there may be unanticipated situations where euthanasia is required, such as when an animal is encountered in the wild that has been fatally injured or has a fatal disease and is experiencing pain and distress. In such instances, the quickest, most humane method should be chosen, and given that the animal is already in pain and distress, priority should be given to euthanizing the animal quickly.

#### **6. How do the guidelines apply to animals in field settings?**

A description of appropriate methods of euthanasia for animals in field settings is provided in Section I Euthanasia of the *CCAC guidelines on: the care and use of wildlife* (2003). While the general principles outlined in the *CCAC guidelines on: euthanasia of animal used in science* still apply, it is recognized that there are inherent differences when research is conducted in field settings. For example, capture and restraint of wildlife is distressful and requires extensive planning based on the species, setting, method, etc., and where chemical methods are used, proper disposal of the contaminated carcass is required. Additionally, veterinarians who do not specialize in wildlife may have little knowledge of wild animals and field research.

**7. The guidelines say that carbon dioxide is not an ideal method of euthanasia for any species, yet it is commonly used for laboratory animals. What are we supposed to do?**

One of the challenges in developing guidelines on euthanasia was the lack of scientific evidence and suitable alternatives in some areas, such as for the use of CO<sub>2</sub>. 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<sub>2</sub>, the guidelines state that there is evidence to show that it is aversive to rats and mice, and therefore it is not a recommended method. However, the document also recognizes that CO<sub>2</sub> is a commonly used method of euthanasia for rodents, particularly when large numbers are involved. For this reason, the guidelines do not prohibit the use of CO<sub>2</sub>, but state that it should not be used where other methods are practical for the experiment and the species. The guidelines then elaborate on current best practice for situations where it has been determined that the use of CO<sub>2</sub> is justified: 1) using a gradual-fill rate of less than 30% and greater than 20% of the chamber volume per minute; and 2) anesthetizing the animals prior to the use of CO<sub>2</sub> where practical.

**8. The guidelines do not include T-61 as an acceptable method. What if there is no suitable alternative?**

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*, vol. 1 (CCAC, 1993) indicated problems associated with improper administration, and this has been further explained in the current guidelines document. The *CCAC 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.

**9. How do the guidelines apply to unhatched eggs?**

Scientific work with embryos (only) need not be described in protocols to be approved by ACCs, unless the institution in question and its ACC choose to review such protocols. For those seeking guidance, acceptable methods of euthanasia for embryonic birds where the shell has been breached include overdose of anesthetic and decapitation (Close et al., 1997). Instantaneous mechanical disruption through maceration is used for euthanasia of unhatched eggs in a hatchery setting, and the use of these devices in a laboratory may be appropriate (Close et al., 1997; EFSA, 2005). Freezing of eggs is a common method; however, it should not be used for the latter third of the incubation period and death must be confirmed by decapitation or some other suitable method. There appears to be emerging evidence that indicates precocial oviparous species are conscious at hatching and during the last few days prior to hatching, and this should be considered when developing the protocol.

**10. Recent publications support use of rapid cooling for certain types of fish. Can the guidelines be overruled by such publications?**

While it is essential that animal users stay current with respect to the scientific progress related to euthanasia methods and procedures, it is equally important that new developments be critically assessed, particularly in areas where few studies have been published. Any proposed changes should be discussed with experienced investigators, veterinarians, the ACC, and others who might have insight into the value of the research and the implications of the proposed changes.

With regard to using rapid cooling as a method for euthanizing certain types of fish, a recent publication supporting this offers an isolated result which is extrapolated to a broader application. For such publications, there should be a critical examination of the methodology and questioning of any anomalies in the results that contrast other bodies of research.

**11. Encouraging the use of isoflurane prior to carbon dioxide could present a human safety hazard. How can this be addressed?**

Gaseous anesthetics such as isoflurane do present health hazards to humans if not properly scavenged. Therefore proper equipment and procedures must be in place prior to use of these chemicals.