Canadian Council on Animal Care *in science*

# CCAC guidelines on:

## euthanasia of animals used in science

This document, the *CCAC guidelines on: euthanasia of animals used in science*, has been developed by the *ad hoc* subcommittee on euthanasia of the Canadian Council on Animal Care (CCAC) Guidelines Committee.

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# TABLE OF CONTENTS

# *euthanasia of animals used in science*

## 1 PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer review agency responsible for setting and maintaining standards for the ethical use and care of animals used in science throughout Canada. The CCAC publishes guidelines on the general care and use of animals in science, as well as on issues of current and emerging concern.

This document aims to provide 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. This guidelines document supersedes Chapter XII – Euthanasia, *Guide to the Care and Use of Experimental Animals*, vol. 1 (CCAC, 1993), which should no longer be used with regard to specific information about methods of euthanasia.

CCAC guidelines documents are developed by subcommittees of experts, based on available scientific evidence and expert opinion, and in response to needs identified within the scientific community, advances in laboratory animal care and procedures carried out on animals, and the needs of the CCAC Assessment Program. When suitable guidance material is available within one or two guidance documents from other jurisdictions, the CCAC will use those documents as the major references for developing guidelines and provide further information and references to facilitate the use of that guidance within the Canadian context. In such cases, the reference documents are an integral component of the information provided, and should be consulted for additional information.

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 (Demers et al., 2006) and the two international reference documents on euthanasia recommended by ICLAS:

- the American Veterinary Medical Association (AVMA) *Guidelines on Euthanasia* (2007) http://www.avma.org/issues/animal_welfare/euthanasia.pdf; and

- *Recommendations for euthanasia of experimental animals,* Part 1(1996) and Part 2 (1997); http://www.lal.org.uk/pdffiles/LA1.pdf; http://www.lal.org.uk/pdffiles/LA2.pdf

This CCAC guidelines document consists of:

- a list of general guiding principles on euthanasia agreed upon by the ICLAS Working Group on Harmonization (Demers et al., 2006), with some modification to fit the Canadian context (see Section 3);

- an overview of acceptable methods of euthanasia for common species used for research, teaching and testing based primarily on the two major reference documents (see Section 4); and

- information on other methods of euthanasia that are not considered best practice but that may be acceptable for specific purposes providing they comply with the general guiding principles outlined in Section 3 and receive the approval of the animal care committee reviewing the application (see Section 5).

Information concerning the euthanasia of wildlife, fish and farm animals can be found in the relevant sections of the respective CCAC guidelines (CCAC, 2003, 2005, 2009).

The professional judgment of the veterinarian, in consultation with the ACC, is required when using any procedures for euthanasia. For information on the possible impacts of various methods of euthanasia on research results, see the addendum, *Additional Information on Effects of Methods of Euthanasia on Research Results*.

These guidelines are intended to provide assistance in the implementation of Russell and Burch's Three Rs (replacement, reduction and refinement) for animals used in science (Russell and Burch, 1959). The CCAC recognizes that guidelines with the term 'should' may be subject to interpretation by properly constituted ACCs, and that in some cases, an ACC may accept a different standard of practice on the basis of adequate justification and provided that the principles of the Three Rs are implemented to the greatest extent possible. This discretion is not extended to any other parties. Where regulatory requirements are involved or where the CCAC considers that no lower standard of practice could be accepted, the term 'must' has been used.

While this guidelines document has been prepared with the greatest possible thoroughness and is as comprehensive as possible, it is not meant to be an instruction manual. 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.

# SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT

## 3. GENERAL GUIDING PRINCIPLES

**Guideline 1:**

Whenever an animal's life is to be taken, the animal must be treated with the highest degree of respect.

*p. 7*

**Guideline 2:**

When performing euthanasia, the intention should be to make the animal's death as distress-free and painless as possible. Therefore, the method likely to cause the least distress and pain to the animal should be selected, consistent with the nature of the experimental protocol.

*p. 7*

**Guideline 3:**

Euthanasia should result in rapid loss of consciousness, followed by respiratory and cardiac arrest and ultimate loss of all brain function.

*p. 7*

**Guideline 4:**

Euthanasia should aim to minimize any pain and distress experienced by the animal prior to loss of consciousness. When appropriate, restraint should be used in such a manner that pain and distress associated with the entire process are minimized.

*p. 8*

**Guideline 5:**

Methods used for euthanasia must be appropriate for the species, age and health status of the animal.

*p. 8*

**Guideline 6:**

Death must be verified following euthanasia and prior to disposal of the animal.

*p. 9*

**Guideline 7:**

Personnel responsible for carrying out the euthanasia must be trained to carry it out in the most effective and humane manner; recognize signs of pain and distress in relevant species; and recognize and confirm unconsciousness, and subsequently death, in relevant species.

*p. 9*

**Guideline 8:**

Human psychological responses to euthanasia should be taken into consideration when selecting the method of euthanasia, but should not take precedence over animal welfare considerations.

*p. 10*

**Guideline 9:**

Animal care committees are responsible for approval of the method of euthanasia for any study involving the use of animals. This includes euthanasia as part of the experimental protocol, as well as euthanasia for animals found to be experiencing unrelievable pain and distress or approaching previously agreed endpoints.

*p. 10*

**Guideline 10:**

A veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little research has been done on euthanasia of that species.

*p. 11*

## 4. OVERVIEW OF ACCEPTABLE METHODS OF EUTHANASIA

**Guideline 11:**

Inhalant anesthetic agents should be administered to induce rapid and controlled effects under controlled conditions with calibrated equipment.

*Subsection 4.1.1.1 Inhalation anesthetics, p. 15*

## 5. CONDITIONALLY ACCEPTABLE METHODS

**Guideline 12:**

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

*Subsection 5.1 Carbon dioxide, p. 18*

**Guideline 13:**

If carbon dioxide use is required for non-anesthetized rodents, a gradual-fill rate of less than 30% and greater than 20% of the chamber volume per minute should be used.

*Subsection 5.1 Carbon dioxide, p. 19*

# 2 INTRODUCTION

Experimental animals are killed for various reasons. These include the need for cells or tissues for in vitro research; for blood, tissues or other samples at certain stages of a study or at the end of a study; for veterinary pathology or diagnostics; to prevent unavoidable pain and distress when the approved endpoint is reached; and to cull animals that are no longer needed (e.g., from a breeding program) when no other use consistent with the tenet of the Three Rs can be found for them.

Whenever an animal is killed in the course of research, teaching, testing or production for scientific purposes, it must be done with respect and in a way that ensures the death is as painless and free of distress as possible. In the use of animals in science, it is essential that the scientific community take on the mantle of responsibility for applying scientific judgment and up-to-date knowledge to ensure that this is achieved.

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 (CNS) to ensure immediate insensitivity to pain, and that steps are taken to minimize distress in the animal prior to the procedure.

Scientific information on humane methods of killing animals is available for certain species, strains, physiological states (e.g., neonatal or pregnant) and situations; however, conclusive information is not available for all species and situations. In addition, there can be stressful effects on the morale of staff and personnel involved in procedures for euthanasia. The application of this guidelines document therefore requires the following:

- professional judgment and technical competence to make an assessment based on both the scientific requirements of the study and the welfare of the animals;
- an understanding of the animal, its behaviour and physiology;
- an understanding of the death process;
- an understanding of the environmental impact of the method of euthanasia used and disposal of the carcass;
- an understanding of the sensitivities of personnel; and
- an understanding of the concerns of the general public.

In line with the *CCAC Ethics of Animal Investigation* (CCAC, 1989), the Three Rs should be applied to considerations relating to euthanasia. Reduction of animal use can be achieved by considering the following scenarios for animals scheduled for euthanasia:

- assigning animals to other studies, where possible; however, the overall level of pain and distress experienced during the animal's lifetime must be taken into consideration, and an individual animal should

only be assigned to one study at category of invasiveness C or higher and euthanized following this study; and

- minimizing the numbers of animals required for a study by controlling variability and taking measures to ensure the manner in which animals are euthanized in the study are consistent. Both the reproducibility and variability of scientific data collected in an experiment involving animals can be affected by a number of factors, including the manner in which the animals are killed (Reilly, 1998).

Refinement of animal care and use should be a continuous process; procedures for euthanasia should be reviewed when new information becomes available. It is essential that animal users stay current with respect to the scientific progress relating to these procedures and critically assess new developments.

ACC members must be encouraged to educate themselves regarding the euthanasia process before reviewing protocols. Adequate training, guidance, resource material for reference, and previous exposure to the process, as well as experience with different methods of euthanasia used, should be accessible by all ACC members.

# 3 GENERAL GUIDING PRINCIPLES

The CCAC has adopted the general principles for euthanasia agreed on by the ICLAS Working Group on Harmonization (Demers et al., 2006). Some modifications have been made to the original wording of these principles to use them as guidelines for investigators and ACCs within Canada. The explanation given below each of the guidelines provides further clarification in the adoption of these principles within the CCAC Program.

**Guideline 1:**

Whenever an animal's life is to be taken, the animal must be treated with the highest degree of respect.

The *CCAC guidelines on: institutional animal user training* emphasizes the importance of sustaining an institutional culture of respect for animal life (CCAC, 1999). The respect for animal life and the philosophy of compassion described within this document should guide the actions of investigators, ACCs and animal care staff throughout the animal's life (including its euthanasia).

All proposed methods for euthanasia of animals, including emergency euthanasia, must be submitted for review and approval by an ACC. During the review of an animal use protocol, the ACCs ensure the intended use of the animals is ethical, and where an animal is to be killed, there is justification to ensure animal life is not wasted and the use of each animal is maximized (for example, by coordinating between different researchers in an institution requiring various tissues before euthanasia).

**Guideline 2:**

When performing euthanasia, the intention should be to make the animal's death as distress-free and painless as possible. Therefore, the method likely to cause the least distress and pain to the animal should be selected, consistent with the nature of the experimental protocol.

These points are emphasized in CCAC guidelines and in the Canadian Veterinary Medical Association (CVMA) position statement on euthanasia (http://canadianveterinarians.net/ShowText.aspx?ResourceID=34). The CVMA position statement on euthanasia emphasizes that "the animal must be rendered irreversibly unconscious as rapidly as possible with the least possible pain, fear, and anxiety." In addition, the Canadian Association of Laboratory Animal Medicine (CALAM) *Standards of Veterinary Care* (http://www.calam-acmal.org/pdfs/StandardsVetCare.pdf) states that "the veterinarian must have the responsibility and authority to assure that … euthanasia is administered as according to current veterinary standards to relieve unnecessary pain or suffering."

**Guideline 3:**

Euthanasia should result in rapid loss of consciousness, followed by respiratory and cardiac arrest and ultimate loss of all brain function.

The most important criterion of acceptance of a method of euthanasia as humane is that it has an initial depressive action on the CNS to ensure loss of consciousness prior to any effects that may otherwise be associated with pain and/or distress.

**Guideline 4:**

Euthanasia should aim to minimize any pain and distress experienced by the animal prior to loss of consciousness. When appropriate, restraint should be used in such a manner that pain and distress associated with the entire process are minimized.

Expression of pain and distress is limited or very subtle for many species, and assessment of these states can be imprecise. Nonetheless, every effort should be made to recognize and assess the responses of individual animals, to make informed judgments about these, and to take appropriate actions based upon the individual situation.

Provision of an appropriate environment favouring a calm and painless death should be the goal in each situation. Whenever possible, animals should be euthanized in their home environment. If animals are moved away from their home environment for prolonged periods, they should have access to food and water until the procedure for euthanasia occurs. Animals that are not group housed with familiar conspecifics should not be mixed prior to euthanasia. Mixing of unfamiliar animals can be extremely stressful.

Veterinarians and/or experienced animal technicians should be consulted regarding the impact of having other animals present during euthanasia. The impact of this practice varies between species and with circumstances. Considerations should be given to circumstances where the method of euthanasia itself, or its application, may result in audible, visual or olfactory alarm signals that might negatively affect other animals in the vicinity. Exposure to alarm signals of other animals may cause stress in some species, e.g., rats (Kikusui et al., 2001), cattle (Boissy et al., 1998) and fish (Toa et al., 2004). While use of separate rooms is preferred, a separately ventilated hood or cabinet is also acceptable. Ideally, equipment should be thoroughly cleaned between animals to ensure animals are not exposed to residues that may contain olfactory alarm signals.

Groups of familiar animals should be euthanized together (at the same time) and separate from other animals. Isolation can result in a stress response for some social species such as sheep (Lowe et al., 2005) and cattle (Boissy and Le Neindre, 1997), and removal of known animals can result in behavioural and physiological changes in rats (Kask et al., 2001). The presence of familiar animals has a role in decreasing negative responses to stressors in social species such as rats (Kiyokawa et al., 2004; Sharp et al., 2002), and it is thought that grouping familiar rats is beneficial during euthanasia (Patterson-Kane et al., 2004).

Many methods of euthanasia require some form of restraint of the animal. Therefore, personnel should be competent in handling the animals in an empathetic and firm manner to minimize pain and distress. Where the restraint may cause these states, the use of tranquillizers, sedatives or anesthetics must be considered in the protocol, provided any additional restraint required or administration of the agent does not cause more pain or distress to the animal than restraint alone.

**Guideline 5:**

Methods used for euthanasia must be appropriate for the species, age and health status of the animal.

In some cases, particular methods of euthanasia are employed to maintain compatibility with previously published results. The justification for the continued use of any method of euthanasia should be reviewed

by the ACC in consultation with the investigator and the attending veterinarian. In cases where more humane methods become available, they should be evaluated (e.g., through pilot studies) to assess their compatibility with the scientific goals of the study.

With neonates, particular care should be taken in choosing the method of euthanasia and the procedures leading up to euthanasia, such as removal from the mother, handling and restraint. Neonates should be euthanized immediately after removal from the mother, unless an alternate experimental procedure has been approved by the ACC. When separated from their mother, pups should be provided with supplemental heating in a manner that does not cause injury to them. A more detailed discussion on the euthanasia of neonates is provided in Section 6.

**Guideline 6:**

Death must be verified following euthanasia and prior to disposal of the animal.

An acceptable methods of euthanasia renders the animal unconscious and insensitive to pain and other adverse effects; however, there must also be assurance of the subsequent death of the animal. Only when there is assurance that blood is no longer being delivered to the brain, and respiration and reflex activity have ceased, should the animal be considered dead.

For some methods of euthanasia (such as $CO_2$ inhalation, stunning using a captive bolt or cervical dislocation), death of the animal should be ensured without it regaining consciousness through application of a second step (e.g., exsanguination, cervical dislocation, decapitation or opening the chest) after application of the primary method of euthanasia.

**Guideline 7:**

Personnel responsible for carrying out the euthanasia must be trained to carry it out in the most effective and humane manner; recognize signs of pain and distress in relevant species; and recognize and confirm unconsciousness, and subsequently death, in relevant species.

All personnel performing procedures for euthanasia must be trained and competent to ensure that euthanasia is carried out in the most humane manner. The procedure must be done in a competent manner with professionalism and respect. Training should include

- recognition of animal pain and distress using behavioural measures;
- proper methods of handling and restraining the animal;
- proper application of the method of euthanasia and use of equipment;
- recognition and assessment of unconsciousness;
- methods of ensuring the death of the animal; and
- recognition and confirmation of death.

Specific training is also needed if the individuals performing euthanasia will be using controlled drugs.

The CCAC experimental animal user training modules (http://www.ccac.ca/en/CCAC_Programs/ETCC/Intro-coretopics-Web11.htm) provide basic theoretical training; however, this must be supplement-

ed with detailed hands-on training provided by the institution, usually by the institution's veterinary personnel or senior technical personnel. Records of training, including training for different methods of euthanasia, should be maintained in a central database. Personnel must be supervised until their competency to perform the particular method has been assured.

**Guideline 8:**

Human psychological responses to euthanasia should be taken into consideration when selecting the method of euthanasia, but should not take precedence over animal welfare considerations.

There may be emotional and psychological effects on the people performing the euthanasia, and on observers, that must be respected and taken into consideration. In research laboratories, staff may become attached to the animals and experience uneasiness at having to euthanize them at the end of a study. It is important to recognize that regular exposure to the task can affect different people in different ways. Some people may raise psychological defense mechanisms that could result in reduced ability to empathize or less respectful handling of the animals. Others may find that regular conduct of the procedure increases their confidence and competence and reduces their own stress, thus improving the performance of the procedure. A number of steps can be taken to minimize any negative impact on personnel performing euthanasia. Positive measures include ensuring that people are skilled in the techniques, that they have a good understanding of the physiological events associated with dying (assurance of unconsciousness, reasons for body movements, etc.), and that they are using the most esthetic techniques compatible with the welfare and scientific imperatives. Moreover, those involved should willingly consent to carry out euthanasia and should not be pressured in any way.

A forum for open discussion of an individual's concerns about euthanasia and support for these individuals should be available. Any person who feels uncomfortable with a particular method of euthanasia, or killing animals in general, should discuss it with his/her supervisor or the veterinarian and should not be "made" to carry out the procedure.

**Guideline 9:**

Animal care committees are responsible for approval of the method of euthanasia for any study involving the use of animals. This includes euthanasia as part of the experimental protocol, as well as euthanasia for animals found to be experiencing unrelievable pain and distress or approaching previously agreed endpoints.

ACCs are responsible for the approval of all aspects of a protocol as it relates to animal care and use. Any protocol involving euthanasia should include a description and justification of the methods to be used to kill the animals humanely. ACCs may be required to consider the use of methods which are not the preferred method of euthanasia of the species, but at all times must ensure that the most humane method is used, consistent with meeting the scientific goals of the protocol. As part of the animal use protocol, records of species, age of animals and method of euthanasia employed should be maintained.

The use of any method should be considered on a case-by-case basis according to its appropriateness in a given situation, taking the scientific literature and scientific goals of the research into account, and in consultation with the attending veterinarian, researcher and animal care staff as appropriate. ACC members should have the opportunity to observe the methods of euthanasia that they are being asked to approve, in order to fully understand the implications of the procedures on the animals and the impacts on the personnel required to kill the animals. Consideration should also be given to the health and safety aspects of

certain procedures (e.g., use of gases, physical injury, ergonomics) and the requirements for storage and use of any controlled substances.

It should be noted that for practical reasons, not all methods of euthanasia and situations have been described within this document.

**Guideline 10:**

A veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little research has been done on euthanasia of that species.

Investigators should discuss the proposed method of euthanasia with a veterinarian knowledgeable about the species in question, to ensure that the selected method of euthanasia minimizes the potential for animal pain and distress. Consultation with a veterinarian may also assist in selection of a method of euthanasia that has minimal negative implications for the scientific goal of the study (for information on possible effects of methods of euthanasia on research results, see the addendum).

# 4 OVERVIEW OF ACCEPTABLE METHODS OF EUTHANASIA

Table 1 provides an overview of methods of euthanasia that are considered acceptable, based on recommendations made in the major reference documents and additional supporting references (Section 8). The table is arranged by animal type according to biologically related groups of animals. In this table, 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.

Other methods of euthanasia may be acceptable when used on anesthetized or unconscious animals (see Section 5 and the addendum). Use of any method of euthanasia should be based on professional judgment with consideration of the species, age, circumstances, expertise and equipment available, and the scientific objectives. This should involve a cost-benefit analysis which considers both the scientific goals of the study and the welfare of the animals involved.

More detailed information, including the impact of these methods on research results, can be found in the addendum. Other methods which may be used if the acceptable methods are likely to interfere with research results are also detailed in the addendum; however, use of these methods requires specific justification to the ACC.

Acceptable methods listed in Table 1 are for euthanasia of animals used for experimental purposes, where higher standards of care should be the norm. Additional information for field studies, fish, and agricultural research is provided in the *CCAC guidelines on: the care and use of wildlife* (CCAC, 2003), *CCAC guidelines on: the care and use of fish in research, teaching and testing* (CCAC, 2005), and *CCAC guidelines on: the care and use of farm animals in research, teaching and testing* (CCAC, 2009).

## Table 1 Summary Chart of Acceptable Methods of Euthanasia for Experimental Animals

| Classification and Common Name | Acceptable Methods | Details and Cautions |
|---|---|---|
| **Class *Amphibia* (Amphibians)** | | |
| Frog, Toad | Immersion or injection of buffered tricaine methane sulfonate (TMS; also known as MS222, tricaine) | Section 4.1.1 & Addendum |
| | Immersion or injection of benzocaine | Section 4.1.1 & Addendum |
| | SC injection of barbiturates into lymph sac | Section 4.1.1 & Addendum |
| | Overdose of inhalant anesthetics (for species that do not breath hold), followed by another method to ensure death | Section 4.1.1 & Addendum |

| Classification and Common Name | Acceptable Methods | Details and Cautions |
|---|---|---|
| **Class *Reptilia* (Reptiles)** | | |
| Turtle, Snake, Lizard | IV or IP injection of barbiturates | Section 4.1.1 & Addendum |
| | Penetrating captive bolt (for larger species) | Section 4.1.2.1 & Addendum |
| **Class *Osteichthyes* (Bony Fishes)**<br>**Class *Chondrichthyes* (Cartilaginous Fishes)** | | |
| Fish | See also *CCAC guidelines on: the care and use of fish in research, teaching and testing* | |
| | Immersion or injection of buffered tricaine methane sulfonate (TMS; also known as MS222, tricaine) | Section 4.1.1.3 & Addendum |
| | Benzocaine[1] | Section 4.1.1.3 & Addendum |
| | Etomidate[1]<br>Metomidate (also known as Marinil™ ) | Section 4.1.1.3 & Addendum |
| | Clove oil[1] | Section 4.1.1.3 & Addendum |
| | Maceration (for fish less than 2cm in length) | Section 4.1.2.2 & Addendum |
| **Class *Aves* (Birds)** | | |
| Chicken, Pigeon, etc. | IV or IP injection of barbiturates with local anesthetic | Section 4.1.1.2 & Addendum |
| | Inert gases (Ar, $N_2$) for poultry | Section 4.1.1.1 & Addendum |
| | Overdose of inhalant anesthetics (for species that do not breath hold), followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |
| | Captive bolt concussion stun/killing for poultry only | Section 4.1.2.1 & Addendum |
| **Class *Mammalia* (Mammals)** | | |
| Order *Rodentia*<br>Mouse, Rat, Hamster, Gerbil, Guinea Pig | IP injection of buffered and diluted barbiturates with local anesthetic | Section 4.1.1.2 & Addendum |
| | Overdose of inhalant anesthetics (for species that do not breath hold), followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |
| Order *Lagomorpha*<br>Rabbit | IV injection of barbiturates | Section 4.1.1 & Addendum |
| | Captive bolt | Section 4.1.2.1 & Addendum |
| | Overdose of inhalant anesthetics, followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |

[1] Currently only TMS and metomidate are registered for veterinary use in Canada for fish that may be consumed by humans; investigators are individually responsible for the use of other anesthetic agents that have not been approved for such use.

| Classification and Common Name | Acceptable Methods | Details and Cautions |
|---|---|---|
| Order *Carnivora*<br>Family *Felidae*<br>Cat | IV injection of barbiturates | Section 4.1.1.2 & Addendum |
| | Overdose of inhalant anesthetics, followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |
| Order *Carnivora*<br>Family *Canidae*<br>Dog | IV injection of barbiturates | Section 4.1.1.2 & Addendum |
| | Overdose of inhalant anesthetics, followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |
| Order *Carnivora*<br>Family *Mustelidae*<br>Ferret, Skunk | IP injection of barbiturates with local anesthetic | Section 4.1.1.2 & Addendum |
| | Overdose of inhalant anesthetics (for species that do not breath hold), followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |
| Order *Artiodactyla*<br>(Hoofed animals)<br>Ruminants<br>Sheep, Cattle, Goats | IV injection of barbiturates | Section 4.1.1.2 & Addendum |
| | Penetrating captive bolt or free bullet, followed by exsanguination or destruction of the brain | Section 4.1.2.1 & Addendum |
| Order *Artiodactyla*<br>(Hoofed animals)<br>Swine | IV injection of barbiturates (or IP injection with local anesthetic) | Section 4.1.1.2 & Addendum |
| | Penetrating captive bolt, followed by exsanguination or pithing | Section 4.1.2.1 & Addendum |
| | Electrical stunning, followed by exsanguination or pithing | Addendum |
| | Overdose of inhalant anesthetics, followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |
| | Argon (under tightly controlled conditions) | Section 4.1.1.1 & Addendum |
| Order *Perissodactyla*<br>(Hoofed animals)<br>Horse, Donkey | IV injection of barbiturates | Section 4.1.1.2 & Addendum |
| | Penetrating captive bolt, followed by exsanguination or pithing | Section 4.1.2.1 & Addendum |
| Order Primates<br>(Non-human primates)<br>Monkeys | IV injection of barbiturates | Section 4.1.1.2 & Addendum |
| | Overdose of inhalant anesthetics, followed by another method(s) to ensure death | Section 4.1.1.1 & Addendum |
| Wildlife<br>Free-ranging mammals<br>Free-ranging birds | See *CCAC guidelines on: the care and use of wildlife* | |
| Marine Mammals | Barbiturates | Section 4.1.1.2 & Addendum |

SC – subcutaneous; IV – intravenous; IP – intraperitoneal

## 4.1 Additional Recommendations for Acceptable Methods

These additional recommendations have been made to address some of the different interpretations of published information within the reference documents, and to alert investigators, laboratory animal veterinarians and ACC members to new research data.

As noted in Guideline 6, death of the animal must be verified at the end of the procedure, irrespective of the method of euthanasia used.

### 4.1.1 Chemical methods

The chemical methods for humanely killing experimental animals include agents that are inhaled (e.g., gasses and volatile anesthetic agents), injected (e.g., barbiturates) and absorbed through other routes (e.g., TMS).

#### 4.1.1.1 Inhalation anesthetics

**Guideline 11:**

Inhalant anesthetic agents should be administered to induce rapid and controlled effects under controlled conditions with calibrated equipment.

In general, overdose of an inhalation anesthetic agent is an effective method of euthanasia for many species. However, time to death is quite lengthy, and therefore use of a second procedure to ensure death of the animal is recommended once the animal is unconscious as a result of the anesthetic.

Exposure to inhalant anesthetics has been found to be aversive in rodents (Leach et al., 2004; Makowska & Weary, 2009) and may also be stressful for other species (e.g., rodents, dogs, cats, ferrets and rabbit). Use in combination with a sedative may be indicated in situations where administration of the sedative results in minimal stress. In addition, gaseous anesthetic agents present health hazards for humans if they are not properly scavenged. Levels of aversion to inhalant agents are highly species and strain specific. It is important to regularly review the literature and good practice in this field.

Inhalant anesthetics are not appropriate for aquatic species or species that breath-hold.

Any mixture of inert gases (Ar, $N_2$) with atmospheric air or $CO_2$ is acceptable for poultry, provided that $CO_2$ <30% v/v and $O_2$ <2%v/v (EFSA, 2005).

#### 4.1.1.2 Injected anesthetics

Ideally anesthetics should be injected intravenously (IV) unless the animal is too small, in which case intraperitoneal (IP) injection should be performed. When the IP injection route is used, operators should be aware of the need to ensure the pH of drugs formulated for IV administration is not irritating. This is particularly important for barbiturates and TMS.

Barbiturates are an effective method of euthanasia for most species. In smaller species where it is not practical to deliver the barbiturate intravenously, resulting in the decision to use other routes (e.g., IP), the solution should be buffered, diluted, and combined with a fast acting local anesthetic such as lignocaine (also known as lidocaine) immediately prior to injection (Ambrose et al., 2000) to reduce irritation. A veterinarian should be consulted whenever dilutions or combinations of barbiturates with other agents are proposed. Prior sedation may be preferable when the animal cannot be safely or adequately restrained to ensure proper injection procedures. For IV or IP injection in birds, care should be taken not to puncture the air sacs.

Where barbiturates have been used, carcasses must be properly identified for disposal; they must not enter the food or feed chain.

#### 4.1.1.3 Immersion

TMS is a recommended method for amphibians and fish; however, it is acidic and must be buffered (Cakir and Strauch, 2005). Use of immersion methods (such as TMS, benzocaine, etomidate, metomidate and clove oil) must be followed by a physical or chemical method to cause brain death. Immersion methods may be weak or ineffectual on fish which breath-hold or breathe air.

### 4.1.2 Physical methods

Physical methods must only be used by highly competent individuals. A number of physical methods for killing animals have not been included as acceptable methods due to the potential for severe pain and distress if they are incorrectly performed. For further information on these methods, consult Section 5, the addendum and the major reference documents.

#### 4.1.2.1 Mechanical stunning methods

When either penetrating captive bolt or concussion stunning is used, the animals may not die immediately, depending on the degree of injury to the brain. Therefore, it is recommended that, immediately after the procedure, death of the animal be ensured by a second procedure such as exsanguination, delivering compressed air into the cranium, or pithing to damage the deeper parts of the brain and to prevent convulsions (EFSA, 2005).

Penetrating captive bolt stunning, using equipment that is appropriate for the species, is suitable for large animals.

#### 4.1.2.2 Maceration

Maceration is an acceptable method for fish less than 2 cm in length when equipment specifically designed for this purpose is used.

### 4.1.3 Other considerations

A number of other methods for killing experimental animals are acceptable when used on animals already unconscious and insensitive to pain (e.g., already anesthetized or stunned). For example, potassium chloride injection is acceptable only when the animal is already deeply anesthetized.

When an animal is encountered in the wild that has been fatally injured or has a fatal disease and is experiencing pain and distress, the quickest, most humane method should be chosen. However, given that the animal is already in pain and distress, priority should be given to euthanizing the animal quickly. Examples of such emergency killing of an animal might be blunt force trauma, gunshot, potassium chloride injection, etc. Safety measures and relevant laws must be respected. In addition, any animal euthanized in the field which may contain residues of toxic euthanasia chemicals should be disposed of in such a manner that it does not enter the food chain.

# 5 CONDITIONALLY ACCEPTABLE METHODS

Some other methods for killing experimental animals 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 considered 'acceptable methods' as listed in Section 4 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 ACC are used, the conditions of use and training of the personnel involved should be clearly stated in the protocol. Some such additional methods are listed in Table 2 and included in the addendum.

**Table 2 Conditionally acceptable methods**

| Species | Methods | Details and Cautions |
|---|---|---|
| Fish | Concussion (emergency killing for other species) | Section 5.5 & Addendum |
| Birds | $CO_2$ | Section 5.1 & Addendum |
| | Cervical dislocation | Section 5.6 & Addendum |
| | Decapitation | Section 5.7 & Addendum |
| | Maceration (chicks within 2 days of hatching only) | Addendum |
| Rodents | $CO_2$ | Section 5.1 & Addendum |
| | Argon/Nitrogen | Section 5.2 & Addendum |
| | Cervical dislocation | Section 5.6 & Addendum |
| | Decapitation | Section 5.7 & Addendum |
| Rabbits | Cervical dislocation | Section 5.6 & Addendum |
| | T-61 | Section 5.3 & Addendum |
| Cats, Dogs | T-61 | Section 5.3 & Addendum |
| Cattle, Sheep, Goats, Horses, Donkeys | Gun shot | Addendum |
| Pigs | $CO_2$ | Section 5.1 & Addendum |

## 5.1 Carbon dioxide

Carbon dioxide is not an ideal method for humanely killing any species (Hawkins et al., 2006). The effects of $CO_2$ exposure have only been examined in a small number of species. For species not discussed below, further study is required before recommendations can be made.

Due to the formation of carbonic acid when $CO_2$ dissolves in water, it is not an acceptable method of euthanasia for aquatic species, including fish and amphibians (CCAC, 2005). In addition, brain electrical activity is sustained in fish exposed to $CO_2$ (Kestin et al., 1995; Robb et al., 2000). It is also unacceptable for breath-holding species such as lagomorphs, reptiles and diving species, and for species that have been shown to exhibit significant aversion to $CO_2$ levels capable of stunning and killing, such as pigs (Raj and Gregory, 1995) and mink (Cooper et al., 1998). While poultry species will enter chambers containing $CO_2$ concentrations sufficient to stun and kill in order to obtain food or social rewards, they have been observed to exhibit behavioural signs of pain and distress (Gerritzen et al., 2000) and aversion (Webster and Fletcher, 2004). Because these results are difficult to interpret in terms of animal welfare, $CO_2$ should not be used as a sole agent of euthanasia where other methods can be practically employed.

$CO_2$ is a commonly used method of euthanasia of rodents, particularly when large numbers are involved, for purposes such as procurement of tissue and for killing animals at the end of a study. The remaining recommendations in this section are aimed primarily at rodents, and more specifically rats. Fewer studies have been carried out concerning the use of $CO_2$ for the euthanasia of mice; however, it appears that mice show similar aversion thresholds as rats (Makowska et al., 2009).

The ethics of any use of $CO_2$ for killing animals must be reviewed thoroughly by the ACC, taking into account current scientific information in this rapidly evolving field. Competency of the person(s) performing the procedure must be assured, and the procedure must be done according to a strict and written SOP, with written records of use. Regular post-approval monitoring review is necessary.

Recommendations within this section are based on the report of the Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals, held February 27-28, 2006 at the University of Newcastle upon Tyne, UK (http://www.lal.org.uk/workp.html), the *Report of the ACLAM Task Force on Rodent Euthanasia* (http://www.aclam.org/print/report_rodent_euth.pdf), and the *Review of Schedule 1 of the Animals (Scientific Procedures) Act 1986 – Appropriate Methods of Humane Killing* (http://www.apc.homeoffice.gov.uk/reference/schedule-1-report.pdf).

**Guideline 12:**

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

Both pre-fill (filling a chamber with $CO_2$ before animals are placed into it) and gradual-fill (placing animals into a chamber containing air and then introducing $CO_2$ at a defined rate; see below) can cause welfare problems in terms of pain and/or distress (Hawkins et al., 2006).

Pre-fill $CO_2$ methods for rodents and other mammals result in increased potential for producing pain. Rats and mice show aversion to high concentrations of $CO_2$ (Leach et al., 2002; Niel and Weary, 2007; Makowska et al., 2009). Studies involving humans indicate that $CO_2$ at > 50% results in pain in the eyes (Feng and Simpson, 2003; Chen et al., 1995) and nasal mucosa (Anton et al., 1992; Danneman et al., 1997). Therefore it has been suggested that rats placed into a chamber containing a high concentration of $CO_2$ (above 50%), will experience at least 10-15 seconds of pain in the upper airways prior to the loss of consciousness (Hawkins et al., 2006).

Gradual-fill $CO_2$ exposure is also aversive to rodents and causes behavioural signs of distress prior to loss of consciousness (Britt, 1987; Leach et al., 2002; Hawkins et al., 2006; Niel and Weary, 2006, 2007). Aversion to $CO_2$ in rats (Niel and Weary, 2007) and mice (Makowska et al., 2009) occurs at concentrations below those required to produce a 'stinging' sensation in the nasal mucosa due to the formation of carbonic acid. Humans report dyspnea, an unpleasant sensation of breathlessness, at $CO_2$ concentrations that are consis-

tent with aversion in rodents (Dripps and Comroe, 1947; Liotti et al., 2001), leading to the suggestion that dyspnea might be a cause of aversion to lower concentrations of $CO_2$.

Where practical, animals should be anesthetized prior to the use of $CO_2$, preferably using inhalant anesthetics. While inhalant anesthetics, such as isoflurane, have also been shown to cause aversion in rodents (Leach et al., 2002; Makowska and Weary, 2009), during self-exposure to gas anesthetics rats appear to be closer to loss of consciousness at the onset of aversive behaviour than with $CO_2$ and inert gases (Makowska and Weary, 2009). Because animals are exposed to aversive concentrations of gas for a shorter duration, initial induction with inhalant anesthetics appears to be more humane than euthanasia with $CO_2$ alone.

If $CO_2$ is used for euthanasia following inhalational anesthesia, it should be introduced soon after loss of consciousness when the breath rate is still relatively high. Once deep anesthesia with markedly suppressed respiration or even respiratory arrest has occurred, $CO_2$ may take a very long time to cause death.

**Guideline 13:**

If carbon dioxide use is required for non-anesthetized rodents, a gradual-fill rate of less than 30% and greater than 20% of the chamber volume per minute should be used.

Based on the studies published by Niel et al. (2008) and Hornett and Haynes (1984), and confirmed in the reports of the Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals and the ACLAM Task Force on Rodent Euthanasia cited above, the current best practice for carbon dioxide euthanasia of rodents is to first place the animals in the euthanasia chamber, and then introduce 100% $CO_2$ gas at a flow rate 20-30% of chamber volume per minute. Flow rates greater than 30% of chamber volume per minute likely result in pain prior to loss of consciousness (Ambrose et al., 2000), whereas flow rates less than 20% of chamber volume per minute are too slow in causing loss of consciousness. The rate should be monitored using a gas flow meter. Flow rates, and hence concentration of $CO_2$ in the chamber, can be increased once the animals have lost consciousness.

Compressed $CO_2$ gas in cylinders is the only acceptable source of $CO_2$ because the inflow to the chamber can be regulated precisely.

While the addition of $O_2$ has been investigated as a refinement to minimize welfare problems associated with $CO_2$, Hawkins et al. (2006) note that there is insufficient information in the literature to reach a clear conclusion on the appropriate level of $O_2$ addition to $CO_2$ for laboratory animals, and recent reports from the European Food Safety Authority (EFSA, 2005) and the American College of Laboratory Animal Medicine (ACLAM, 2005) discourage the addition of $O_2$. A study by Coenen et al. (1995) suggests that addition of $O_2$ is not contraindicated and may minimize gasping in rodents, and studies by Coenen et al. (2009) and McKeegan et al. (2007) suggest that for chickens, a two-phase euthanasia process involving anesthetizing the birds with a mix of $CO_2$, $O_2$ and $N_2$, followed by a reduction of $O_2$ and an increase in $CO_2$ results in a smooth and gradual induction to unconsciousness that outweighs the negative $CO_2$ experiences. However, results of aversion testing by Kirkden et al. (2008) suggest that there is only a minimal effect of $O_2$ supplementation on rats' avoidance of $CO_2$, and other studies have shown $O_2$ supplementation causes increased lung hemorrhage prior to loss of consciousness (Ambrose et al., 2000; Danneman et al., 1997). It is also possible that high concentrations of $O_2$ prolong consciousness, which may not be desirable.

### 5.1.1 Other factors

When carrying out euthanasia on several groups of animals using the same chamber, the chamber must be flushed with air between groups. $CO_2$ is denser than air and will settle at the bottom of the chamber; therefore, the $CO_2$ concentration will be greater in the chamber than in the surrounding environment.

It is not clear whether the development of neonates is such that inhalant anesthetics cause pain or distress, and further study is required in this area. However, fetal and immature forms are tolerant of hypoxia and hypercapnia, suggesting that time to death may be considerably longer in preweanling animals (Pritchett et al., 2005). Recommended methods for neonates are given in Section 6.2.

## 5.2 Argon/Nitrogen

Argon/nitrogen is only acceptable for use on pigs and poultry, and is conditionally acceptable for other species, including rodents, when scientific justification is provided and approved by the ACC. Breathing profoundly hypoxic atmospheres may be a humane method of euthanasia in some species; however, responses to different gases used to induce hypoxia are highly species specific, such that it is not possible to generalize between species. Argon has been observed to be aversive for rats (Makowska et al., 2008; Niel and Weary, 2007; Leach et al., 2002), as they will not tolerate an argon-filled chamber for long enough to lose consciousness. However, pigs and poultry have been shown to enter lethal concentrations of argon for access to rewards, suggesting that it is not aversive to these species (Webster and Fletcher, 2004; Raj and Gregory, 1995; Raj, 1996). For species that have not been proven to be non-aversive to argon/nitrogen, these mixes should only be used if the animal is under general anasthesia. Argon/nitrogen mixes should not be used with birds and mammals that are resistant to hypoxia (e.g., breath-holding and diving species and neonates).

Oxygen concentrations of less than 2% (>90% argon/nitrogen[1]) are necessary for argon/nitrogen to result in unconsciousness and death. These concentrations are difficult to achieve. Even lifting the top of the cage to place the animal in can increase the oxygen levels sufficiently to prevent unconsciousness from occurring rapidly. This method is only appropriate if performed under controlled conditions where the $O_2$ concentration is known.

## 5.3 T-61

T-61 is not a recommended method for any species. The ACC must review its application and be aware of its mechanism of action when reviewing protocols requesting its use. It should only be used intravenously and at carefully monitored rates of injection according to the manufacturer's recommendations because of the differential rates of absorption and onset of action of the active ingredients when administered by other routes. 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.

## 5.4 Exsanguination

Animals must be unconscious (e.g., deep anesthesia) prior to exsanguination. Sedation prior to exsanguination does not ensure unconsciousness.

## 5.5 Concussion

There may be instances, such as the emergency killing of injured newborn piglets, when a crushing blow to the head is the most rapid and practical method available. When used, it should be carried out in such a manner that the animal is rendered unconscious almost instantaneously. The procedure must be performed by someone with appropriate training, and should be conducted in an area beyond the sensory range of other animals.

1 The effectiveness of argon/nitrogen for euthanasia is based on the depletion of oxygen; an oxygen concentration of less than 2% is required. A cage with a concentration of 90% argon/nitrogen would contain 10% original air, and because the concentration of oxygen in air is 20.9%, the cage would contain approximately 2% oxygen.

## 5.6 Cervical dislocation

Unless it will interfere with the scientific outcome of the study, animals should be anesthetized prior to cervical dislocation. Commercial cervical dislocators/luxators must be used on heavier rats (>200g) and rabbits (>2kg). Manual cervical dislocation should only be performed where the number of animals is relatively low to prevent human error due to fatigue, and should only be performed on birds <3kg, rodents < 200g and rabbits < 1kg. Training is required to ensure that dislocation is cervical and not lower in the vertebral column. It is essential to check that the neck is broken at the end of the procedure by palpation of the vertebrae. If adequate separation is not observed, a backup method, such as decapitation or exposure to high concentrations of $CO_2$, should be used immediately.

## 5.7 Decapitation

Decapitation is considered conditionally acceptable for rodents and birds, as indicated in Table 2. The length of time of residual consciousness experienced by the severed head of an animal following decapitation has been under debate in the scientific literature. While there is evidence that electrical activity persists in the brain for 13-14 seconds following decapitation (Mikeska and Klemm, 1975; Gregory and Wotton, 1990), others have argued that rats are likely to lose consciousness within a much shorter time (Derr, 1991; Holson, 1992). However, the stress of handling and restraint must also be taken into account. The use of anesthesia prior to decapitation would make this an acceptable method of euthanasia.

Decapitation is often used when chemical methods may interfere with research results. Where decapitation is used, blades should be kept very sharp and guillotines should be well maintained and cleaned between uses to prevent transmission of olfactory clues. Personnel performing decapitation must be trained in the proper and safe use of the equipment.

## 5.8 Pithing frogs

Frogs to be rendered brain dead by pithing should first be anesthetized to a surgical plane of anesthesia (loss of blink reflex or toe pinch) by one of the following methods:

- injection of TMS intravenously or intraperitoneally;
- injection of sodium pentobarbital into the dorsal lymph sacs or intracoelomically; or
- immersion in buffered TMS or benzocaine hydrochloride (Reed, 2005).

Pithing should only be performed by trained and competent personnel.

# 6 CONSIDERATIONS RELATING TO FETAL AND NEONATAL EUTHANASIA

Although fetuses are not included in the animal use statistics reported to the CCAC, the CCAC takes a "moral stewardship" approach to the use of animals in science (*CCAC Experimental Animal User Training Core Topics - Module 2, Ethics in Animal Experimentation*, http://www.ccac.ca/en/CCAC_Programs/ETCC/Module02/toc.html) and, as stated in the *CCAC guidelines on: institutional animal user training* (CCAC, 1999), "Institutions must strive through their training programs to sustain an institutional culture of respect for animal life." For this reason, information concerning fetal and neonatal euthanasia is provided below.

## 6.1 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.

## 6.2 Rodent fetuses and neonates

The following recommendations are based on guidelines developed by the ACLAM Task Force on Rodent Euthanasia (http://www.aclam.org/print/report_rodent_euth.pdf). While there is evidence of progressive development of pain pathways in neonates (Johnson et al., 2009; Diesch et al., 2009; Johnson et al., 2005), there are significant differences between species in the development of these pathways, and therefore a precautionary approach should be taken in the application of the method of euthanasia.

There is insufficient scientific information to determine whether neonates administered inhalant anesthetics experience any pain or distress, and further study is required. However, fetal and immature forms are resistant to hypoxia and hypercapnia, and studies by Pritchett et al. (2005) and Pritchett (2009) indicate that time to death using $CO_2$ may be considerably longer in pre-weanling mice and rats than in adults of the same species. Decisions should be made on a case-by-case basis, taking into account current literature and an analysis of the potential impacts on animal welfare in each situation.

### 6.2.1 Rodent fetuses before two-thirds of the gestation period

Neural development at this stage is minimal and nociception is considered unlikely. Euthanasia of the mother or removal of the fetuses should ensure rapid death of each fetus due to loss of blood supply and non-viability of the fetuses at this stage of development. If there is uncertainty regarding the developmental age of the fetus, appropriate steps (as defined in Section 6.2.2) should be followed.

### 6.2.2 Rodent fetuses after two-thirds of the gestation period to birth

As noted by EFSA (2005), during the final third of the gestation, fetuses should be given the same ethical considerations as apply to the fully mature animal. Neural development at this stage supports the likelihood that nociception is present, although the ability to perceive or experience pain has been contested by Mellor and Gregory (2003).

When fetuses are required for study, euthanasia may be induced by chemical anesthetics administered using a route appropriate for the size, stage of development and species. Decapitation with sharp, well maintained scissors or cervical dislocation is an acceptable physical method of euthanasia for fetuses. Animals should be anesthetized prior to freezing, and rapid freezing in liquid nitrogen as a sole method of euthanasia, without prior anesthesia, is not considered to be humane. When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion or perfusion with fixative solutions. Anesthesia may be induced by injection of the fetus with a chemical anesthetic; however, the institutional veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents.

Fetuses at this age are resistant to hypoxia and require extended exposure to inhalant anesthetics, including $CO_2$. Considering results with neonatal mice, exposure to 100% $CO_2$ for at least 60 minutes is necessary to ensure death if $CO_2$ alone is used, without decapitation or exsanguination (Pritchett et al., 2005).

When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother should ensure rapid cerebral anoxia to the fetus with minimal disturbance to the uterine milieu. Recommended methods are anesthesia followed by $CO_2$ exposure, with or without cervical dislocation of the mother. Death of the mother must be verified after euthanasia and prior to disposal. The institutional veterinarian should be consulted for considerations of other methods of euthanasia.

### 6.2.3 Mouse, rat and hamster neonates up to 10 days of age

Acceptable methods of euthanasia include those recommended for adult rodents; however, modification may be necessary and veterinary advice should be sought. For example, for mice and rat neonates, the time to death using $CO_2$ varies substantially with age, and can be considerably longer than for adults of the same species (Pritchett et al., 2005; Pritchett, 2009). Pritchett et al. (2005) also note strain differences in mice neonates exposed to $CO_2$.

Immersion in liquid nitrogen may be used only if preceded by anesthesia. Similarly, anesthesia should precede immersion or perfusion with chemical fixatives.

### 6.2.4 Precocial neonates

Precocial species (e.g., guinea pigs) should be treated as adults due to their advanced development.

# 7 RECOMMENDATIONS FOR RESEARCH WHERE SCIENTIFIC KNOWLEDGE IS LACKING

Some areas where additional scientific information is needed have been identified in this document. When new information on specific techniques that may refine methods of euthanasia supported in this document is presented, it should be considered in order to improve welfare outcomes for the animals.

# 8 REFERENCES

This section provides information concerning the documents on which the *CCAC guidelines on: euthanasia of animals used in science* is based. Section 8.1 describes the two major reference documents and sections 8.2 and 8.3 list additional supporting references which were used to develop recommendations in areas where new scientific evidence has emerged.

## 8.1 Major Reference Documents

### 8.1.1 AVMA Guidelines on Euthanasia (2007) http://www.avma.org/issues/animal_welfare/euthanasia.pdf

The *2000 Report of the AVMA Panel on Euthanasia* was prepared at the request of the American Veterinary Medical Association Council on Research by the Panel on Euthanasia that convened in 1999 to review and make necessary revisions to the fifth Panel Report, published in 1993. In the 2000 Report, the panel updated information on euthanasia of animals in research and animal care and control facilities; expanded information on ectothermic, aquatic and fur-bearing animals; added information on horses and wildlife; and deleted methods or agents considered to be unacceptable. In 2006 the AVMA Executive Board approved a recommendation that the AVMA convene a panel of scientists at least once every 10 years to review all the literature that scientifically evaluates methods and potential methods of euthanasia for the purpose of producing AVMA guidelines on euthanasia. During the interim years, requests for inclusion of new or altered procedures or agents of euthanasia in the *AVMA Guidelines on Euthanasia* are directed to the AVMA Animal Welfare Committee. Revisions are based on a thorough evaluation of the available science and require Executive Board approval. The first interim revision was approved as the *AVMA Guidelines on Euthanasia* (2007).

### 8.1.2 Recommendations for euthanasia of experimental animals, Part 1 (1996) ***Laboratory Animals*** 30:293-316, and Part 2 (1997) ***Laboratory Animals*** 31:1-32

These documents were prepared for DGXI of the European Commission to be used with Directive 86/609/EEC of 24 November 1986, on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (No L 358, ISSN 0378-6978). They refer especially to Article 2(1) published by the European Commission in October 1995 which defines "humane method of killing" as "the killing of an animal with a minimum of physical and mental suffering, depending on the species." These documents should be used in conjunction with the opinion of the animal welfare panel of the European Food Safety Authority (EFSA), listed below.

## 8.2 Additional Supporting References

**Aspects of the biology and welfare of animals used for experimental and other scientific purposes (2005) *The EFSA Journal* 292:1-136; http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620774242.htm**

This report summarizes the position of the animal welfare panel of the European Food Safety Authority which was asked to consider the scientific evidence for the sentience and capacity of all invertebrate

species used for experimental purposes and of fetal and embryonic forms to "experience pain, suffering, distress or lasting harm." The Panel also considered and made recommendations concerning humane methods of killing animals. This report updates recommendations made in the above reports prepared for Directive 86/609/EEC of 24 November 1986.

**American College of Laboratory Animal Medicine (2005) Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia; http://www.aclam.org/print/report_rodent_euth.pdf**

The ACLAM Task Force report is a response to growing concerns and controversy regarding methods that were commonly used for rodent euthanasia. Three issues were targeted as the focus of the report: euthanasia of fetal and neonatal rodents; the use of $CO_2$ for rodent euthanasia; and the impact of methods of euthanasia on data.

**The Report of the Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals (2006); http://www.lal.org.uk/pdffiles/CO2%20Final%20Report.pdf**

Carbon dioxide continues to be widely used for killing laboratory rodents. However, because of uncertainties about the humaneness of some protocols, and uncertainties about the feasibility of alternate methods of humanely killing laboratory rodents, a meeting was organized in February 2006 in Newcastle UK. The Newcastle meeting was a unique opportunity to bring scientists who have studied $CO_2$ together with experts from related fields, national organizations responsible for guidelines and regulations, representatives from animal welfare organizations, and representatives of animal care personnel. The objectives of the Newcastle meeting were to achieve consensus views on best practice; establish areas of disagreement about the use of $CO_2$; identify research needed to resolve disagreements; identify needs for further research into $CO_2$ euthanasia; meet the immediate need for guidance on $CO_2$ euthanasia; and consider whether there are preferable alternatives.

**Review of Schedule 1 of the Animals (Scientific Procedures) Act 1986 – Appropriate Methods of Humane Killing; http://www.apc.gov.uk/reference/schedule-1-report.pdf**

The UK Animal Procedures Committee was asked in June 2001 to review Schedule 1 (appropriate methods of humane killing) to the Animals (Scientific Procedures) Act 1986. Recommendations made in the report include advice on humane killing of neonatal rodents; use of argon, nitrogen or other inert gases; use of $CO_2$; and weight thresholds for cervical dislocation of rodents.

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# 9 GLOSSARY

**Humane** – conditions which promote physical and behavioral well-being of animals; in the case of euthanasia, humane methods are those which minimize pain and distress and are reliable, reproducible, irreversible, simple, safe and rapid.

**Neonate** – a newborn animal; in terms of euthanasia, different procedures are required for mouse, rat and hamster neonates under 10 days of age, while methods recommended for neonates over 10 days of age are the same as those for adults; methods recommended for all precocial neonates are the same as those for adults.