

EUTHANASIA OF ANIMALS IN SCIENCE

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Objectives

The objectives of this module are:

- to highlight the guiding principles of euthanasia of animals in science;
- to ensure proper handling of animals prior to euthanasia;
- to acknowledge the emotional impact of euthanasia on the people involved; and
- to outline considerations in choosing a suitable method of euthanasia.

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Introduction

Euthanasia means a gentle death. In the context of animals in science, the term is generally used to refer to humane killing, or doing what is humanly possible to minimize pain and distress to the animals, given the circumstances (including the research goals) under which animals' lives are terminated.

Animals in science are euthanized for various reasons, such as:

- to provide cells or tissues for in vitro research;
- to collect blood, tissues, or other samples at the end of a study;
- to do veterinary pathology or diagnostics;
- to prevent unnecessary pain and suffering when the approved endpoint is reached; and
- to terminate the lives of animals when no further use consistent with the Three Rs tenet (i.e., reduction, replacement, and refinement) can be found for them (e.g., at the end of a study, surplus animals generated by a breeding program).

“Whenever an animal is killed in the course of research, teaching, and testing or production for scientific purposes, it must be done with respect and in a way that ensures the death is as painless and distress-free 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.”

CCAC guidelines on: euthanasia of animals in science (CCAC, 2010)

Guiding Principles for Euthanasia

The most important criteria for accepting a method of euthanasia are that:

- 1) it has a rapid initial depressive action on the central nervous system to ensure immediate insensitivity to pain; and
- 2) steps are taken to minimize distress in the animal prior to the procedure.

This is supported by the *Canadian Veterinary Medical Association Euthanasia – Position Statement* (CVMA, 2014), which states: “The Canadian Veterinary Medical Association (CVMA) holds that when animals are euthanized, death must be quick using a method that causes the least possible pain and distress.”

The 10 guiding principles for euthanasia, as stated in the [*CCAC guidelines on: euthanasia of animals in science*](#) (CCAC, 2010) are as follows:

- Whenever an animal's life is to be taken, the animal must be treated with the highest degree of respect.
- 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.
- Euthanasia should result in rapid loss of consciousness, followed by respiratory and cardiac arrest and ultimate loss of all brain function.
- 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.
- Methods used for euthanasia must be appropriate for the species, age and health status of the animal.
- Death must be verified following euthanasia and prior to disposal of the animal.
- 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.
- 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.
- 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.
- 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.

The application of these principles requires professional judgment and technical competence to make an assessment based on both the scientific requirements of the study and the welfare of the animals. It also involves an understanding of the animal, its behaviour, and its physiology, as well as an understanding of the death process, the environmental and ecological impact of both the method of euthanasia used and disposal of the carcass, the sensitivities of other personnel, and the concerns of the general public.

Handling of Animals Prior to Euthanasia

Any handling and restraint of an animal that is necessary for euthanasia should be done in a gentle, careful manner to minimize the animal's fear, distress, and pain. Where restraint may cause fear, distress, or pain to the animal, the use of tranquilizers or sedatives should be considered.

Competency of Personnel

Personnel must be competent in performing the method of euthanasia on the particular animals involved, to ensure it is carried out in the most humane manner and that it is done with professionalism and respect. Competency includes the ability to:

- recognize pain and distress in the animal using behavioural indicators;
- apply appropriate methods of handling and restraining the animal as necessary;
- perform the method of euthanasia using appropriate equipment;
- recognize and assess unconsciousness in the animal;
- perform a secondary method to ensure the death of the animal when necessary; and
- recognize and confirm death.

Practical training is beyond the scope of this module and should be provided separately by the institution (see the [CCAC guidelines on: training of personnel working with animals in science](#) [CCAC, 2015]).

Equipment Used to Perform Euthanasia

Any instruments or devices used for euthanasia of animals should allow for easy observation of the animals and should be professionally designed and kept in good repair to effectively produce rapid unconsciousness and death. Equipment should be cleaned of all animal tissue, blood, and excreta after each use.

Ensuring the Death of the Animal

While any acceptable method of euthanasia rapidly renders the animal unconscious and insensitive to pain, the death of the animal must also be assured. The animal should be considered dead only when there is assurance that blood is no longer being delivered to the brain because the heart has stopped, and all other movements, such as respiration or reflex activity, have ceased. For some methods of euthanasia, this involves two steps: the application of the method producing initial unconsciousness, followed by a procedure to ensure the animal cannot regain consciousness or recover (e.g., exsanguination, opening the chest, decapitation or cervical dislocation after carbon dioxide euthanasia).

Carcass Disposal

All experimental animal tissues and carcasses must be disposed of according to institutional policy and federal, provincial or territorial, and municipal regulations.

Emotional and Psychological Impact on Personnel

There may be emotional and psychological effects on people performing or observing euthanasia that must be respected and taken into consideration. In research laboratories, personnel 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 defence 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 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 aesthetic techniques compatible with the welfare of the animals and the scientific objectives. 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 with performing the procedure, should discuss it with a supervisor or veterinarian.

Choosing a Suitable Method of Euthanasia

Scientific Concerns Relating to the Choice of Euthanasia Method

It is important to consider whether research results will be impacted by a proposed method of euthanasia, as some methods may affect animal tissues. For information on the potential effects of various methods of euthanasia on particular species, see [*Additional information on effects of euthanasia methods on research results: Addendum to the CCAC guidelines on euthanasia of animals in science*](#) (CCAC, 2010).

Proper handling of animals prior to death to avoid stress or fear is also important.

Description of a Number of Acceptable and Conditionally Acceptable Methods

The selection of a method of euthanasia should include consultation with a veterinarian. Before a method of euthanasia is used, it must be approved by the animal care committee during protocol

review. Appropriate records should be kept of euthanasia, including the method and drug used, and the personnel involved.

The *CCAC guidelines on: euthanasia of animals in science* (CCAC, 2010) classifies potential methods of euthanasia as:

- **acceptable** – methods that are simple to perform and consistently produce death, with minimal pain and distress when used on conscious or sedated animals; or
- **conditionally acceptable** – methods that have greater potential for operator error or safety hazards, might not consistently produce humane death, or are not well documented in the scientific literature.

Conditionally acceptable methods may be suitable for use in certain circumstances where there is scientific justification and animal care committee approval, and where there is assurance that competent personnel are available.

Some common methods of euthanasia and brief descriptions of their use are presented here. Details of which methods are acceptable or conditionally acceptable for particular species are provided in the *CCAC guidelines on: euthanasia of animals in science* (CCAC, 2010) and the CCAC's animal-type guidelines.

Chemical Methods – Inhalants

Inhaled euthanasia agents are delivered to the animal, either as vapours (from a liquid) or gases, usually in a closed chamber to avoid human exposure. Vapours and gases must be properly scavenged.

Inhalant Anesthetics

An overdose of a commonly used inhalant anesthetic (e.g., halothane or isoflurane) can be an effective method of euthanasia for many species. However, time to death with inhalant anesthetics is quite lengthy, and therefore, the use of a second procedure to ensure death of the animal is recommended, once the animal is unconscious as a result of the anesthetic. Inhalant anesthetics are not appropriate for aquatic species or species that breath hold. Inhalant anesthetic agents must be administered under controlled conditions with calibrated equipment (i.e., using a vapourizer).

Exposure to inhalant anesthetics has been found to be aversive to rodents and may also be stressful for other species. The level of aversion appears to be species and strain specific, and depends on previous exposure to the anesthetic. Combining the anesthetic with a sedative may be indicated where administration of the sedative helps minimize stress.

Inhalant Gases

Carbon dioxide (CO₂) is commonly used for the euthanasia of rodents, particularly when large numbers of rodents are being euthanized. However, CO₂ is only considered conditionally acceptable for the euthanasia of small laboratory animal species, and should not be used where other methods are practical for the experiment and the species.

The ethics of any use of CO₂ for killing animals must be reviewed thoroughly by the animal care committee, taking into account current scientific information in this rapidly evolving field. When CO₂ euthanasia is used, it must be done according to a standard operating procedure approved by the animal care committee, and the competency of the persons performing the procedure must be assured.

CO₂ is not an acceptable method of euthanasia for:

- aquatic species (fish and amphibians), due to the formation of carbonic acid when CO₂ dissolves in water; there is also evidence of sustained brain electrical activity in fish exposed to CO₂;
- breath-holding species (e.g., lagomorphs, reptiles, and diving species); and
- species that have been shown to exhibit significant aversion to CO₂ levels capable of stunning and killing (e.g., pigs and mink).

Most studies of the effects of CO₂ euthanasia on rodents have been conducted on rats; however, there is evidence that mice show similar aversion thresholds.

Both pre-fill and gradual fill methods of exposure to CO₂ have been shown to be aversive to rodents; however, the current best practice is to first place the animals in the euthanasia chamber, and then introduce 100% CO₂ at a flow rate between 30 and 40% of the chamber volume per minute. Flow rates greater than 40% of chamber volume per minute likely result in pain prior to loss of consciousness, whereas flow rates less than 30% 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 CO₂ in the chamber, can be increased once the animals have lost consciousness.

Where practical, animals should be anesthetized prior to the use of CO₂, preferably using inhalant anesthetics. The use of CO₂ following anesthesia (with isoflurane) is an approved method of euthanasia. While inhalant anesthetics, such as isoflurane, have also been shown to cause aversion in rodents, there is evidence that rats appear closer to loss of consciousness at the onset of aversive behaviour with inhalant anesthetics than with CO₂. However, prior exposure to inhalant anesthetics may increase the animal's aversiveness to them.

If CO₂ euthanasia is to be carried out on different groups of animals using the same chamber, the chamber must be flushed with air between groups. CO₂ is denser than air and will settle at the bottom of the chamber; therefore, the CO₂ concentration will be greater in the chamber than in the surrounding environment.

Chemical Agents – Injectable

Barbiturates

Barbituric acid derivatives are acceptable euthanasia agents for most species if given intravenously at high doses. In smaller species, intraperitoneal administration of barbiturates is acceptable when the intravenous route is not practical and would cause distress; however, barbiturates should be buffered if administered intraperitoneally, to avoid irritation.

Barbiturates act by depressing the central nervous system (anesthetic properties). Concentrated solutions of sodium pentobarbital are the most widely used barbiturates. The Canadian Veterinary Medical Association considers intravenous injection of concentrated barbiturates to be the most humane method of euthanizing companion animals. From the Canadian Veterinary Medical Association's *Euthanasia – Position Statement* (CVMA, 2014): “The intravenous injection of a concentrated barbiturate with prior sedation is widely considered the most humane method of euthanizing companion and many non-domestic animals. It causes a comparatively aesthetic death, is rapid-acting, reliable, and effective.” Records must be kept of all barbiturate use.

T-61

T-61 is an injectable euthanasia agent comprised of three drugs: a local anesthetic, a strong hypnotic, and a paralytic. It is not a recommended method of euthanasia for any species. When approved by the animal care committee after careful review, T-61 must be administered intravenously at the dose and rate recommended by the manufacturer 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.

Chemical Agents – Immersion

Tricaine Methanesulfonate (TMS or MS 222)

Tricaine methanesulfonate is a benzoic acid derivative that is used for anesthesia of fish and amphibians, and can also be used for euthanasia in these species. Stock solutions are dissolved in water (concentration greater than 250 mg/l) and must be buffered. Immersion methods must be followed by a physical or chemical method to cause brain death.

Other immersion methods (e.g., benzocaine, etomidate, metomidate, or clove oil) must also be followed by a second method to ensure death.

Physical Methods of Euthanasia

Whether any physical method is humane strongly depends on the competency of the operator and on the use of properly functioning equipment. The competency of the operator must be assessed in advance. The use of any physical method requires justification to the animal care committee.

Cervical Dislocation

Cervical dislocation is a conditionally acceptable euthanasia method for small rodents, rabbits, and some species of birds. Competency of the operator is essential.

Unless it will interfere with the scientific outcome of the study, animals should be anesthetized prior to cervical dislocation.

Commercial cervical dislocators and luxators must be used for heavier rats (>200 g) and rabbits (>2 kg). Manual cervical dislocation should only be performed when the number of animals to be euthanized is relatively low, to prevent human error due to fatigue, and should only be performed on small birds (<3 kg), rodents (<200 g), and rabbits (<1 kg).

Decapitation

Decapitation is a conditionally acceptable euthanasia method for birds and rodents. Personnel must be competent in proper handling of the animal and in performing the procedure. Guillotines specifically designed for the procedure should be used and kept in good repair to ensure that decapitation is humanely performed. The use of anesthesia prior to decapitation would make this an acceptable method of euthanasia.

Concussion

A concussive blow to the head is a conditionally acceptable euthanasia method and may be appropriate for the emergency killing of small or young animals with a soft skull. Death of the animal must be ensured using a secondary method. The competency of the operator must be assured in advance.

Concussion is a conditionally acceptable method for euthanizing fish, and as a method of emergency killing in other species. When used, it should be carried out in such a manner that the animal is rendered unconscious almost instantaneously. The procedure should be conducted in an area beyond the sensory range of other animals.

Penetrating Captive Bolt

A penetrating captive bolt that is applied by competent personnel, using equipment appropriate for the species, is suitable for the euthanasia of large animals. With accurate placement of the device against the skull of the animal, there is sudden unconsciousness, progressing to death. Immediately after the procedure, a second method should be applied to ensure the death of the animal.

Summary

The euthanasia of animals requires respect for the animal, competency, and an understanding of the many factors to be considered when selecting the most appropriate method. The primary requirements for a humane method are that it causes very rapid (immediate) unconsciousness and subsequent death, no pain is experienced by the animal, and distress to the animal is minimized.

References

Refer to the references in Section 8 of the [*CCAC guidelines on: euthanasia of animals used in science*](#) (CCAC, 2010).