Guidelines for the Humane Euthanasia of Laboratory Animals
The University of Texas at Austin
Institutional Animal Care and Use Committee (IACUC)

These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.

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This document provides information to be used when planning and performing euthanasia of vertebrate animals used for biomedical research or teaching at The University of Texas at Austin. It is organized into three parts:

Section A – General Background
Section B – Recommended Methods
Section C – Technical Comments

Section A – General Background

Definition

The NIH *Guide for the Care and Use of Laboratory Animals* defines euthanasia as "the procedure of killing animals rapidly and painlessly". Techniques used for euthanasia must be chosen to assure that a rapid loss of consciousness will occur, followed shortly by death without pain or significant distress being perceived by the animal.

Humane Considerations

There is a wide variety of animal species used in biomedical research, and specific methods used for each species must be considered based on their anatomy and physiology. However, the general principles for humane euthanasia in all species have been summarized by the International Council for Laboratory Animal Science (2006):

Principles for Animal Euthanasia
http://www.sciencemag.org/cgi/reprint/312/5774/700.pdf

1. Whenever an animal's life is to be taken, it should be treated with the highest respect.
2. Euthanasia should place emphasis on making the animal's death painless and distress-free. The method likely to cause the least pain and distress to the animals should be used whenever possible.
3. Euthanasia techniques should result in rapid loss of consciousness, followed by cardiac or respiratory arrest and ultimate loss of brain function.
4. Techniques should require minimum restraint of the animal and should minimize distress and anxiety experienced by the animal, before loss of consciousness.
5. Techniques used should be appropriate for the species, age, and health of the animal.
6. Death must be verified following euthanasia and before disposal of the animal.

7. Personnel responsible for carrying out the euthanasia techniques should be trained:
   (i) to carry out euthanasia in the most effective and humane manner;
   (ii) to recognized signs of pain, fear, and distress in relevant species; and
   (iii) to recognize and confirm death in relevant species.
8. Human psychological responses to euthanasia should be taken into account when selecting the method of euthanasia, but should not take precedence over animal welfare considerations.
9. Ethics committees should be responsible for approval of the method of euthanasia (in line with any relevant legislation). This should include euthanasia as art of the experimental protocol, as well as euthanasia for animals experiencing unanticipated pain and distress.
10. A veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little species-species euthanasia research has been done.

Gentle, careful handling of subject animals is of the utmost importance during the procedure in order to minimize distress to the animal. Measures should be taken to ensure that euthanasia is performed in a way that minimizes reactions among other animals that may be present. Euthanasia should be performed quickly and efficiently in a procedural area that is separate from rooms in which animals are housed.

When considering the impact of euthanasia on animal well-being, it is important to note that an unconscious animal does not perceive pain. Appropriately conducted procedures that render the cerebral cortex nonfunctional eliminate the perception of pain. Once this initial unconscious state is reached, reflex motor activity may still be observed, but pain is not perceived. This concept can be utilized in two-step approaches that combine an initial anesthetic event (e.g., general anesthesia via isoflurane or tricaine) with a secondary physical method (e.g., decapitation or exsanguination).

Protocol Requirements

Euthanasia is generally performed at the end of a project or, in some cases, at a point where animals would otherwise experience severe or chronic pain or distress that cannot be relieved. Because euthanasia may be needed as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments, protocols should include criteria for monitoring and initiating an early endpoint. This type of pre-planning for potential adverse outcomes will enable a prompt decision to be made by the research staff in conjunction with the veterinarian to ensure that the studies are humane and the objective of the protocol is achieved.

Even when the planned experiment does not include euthanasia, there may be a need to humanely euthanize animals for unanticipated reasons. For this reason, at least one method must be documented for each species used in a protocol.

Euthanasia techniques must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) during review and approval of the submitted protocol application form. Any subsequent change in euthanasia techniques must also be reviewed and pre-approved by the IACUC. The Office for Laboratory Animal Welfare (OLAW) characterizes the method of euthanasia as a significant component of the animal use protocol. **Use of a euthanasia technique that is not described in the approved protocol may be considered significant noncompliance, which can result in protocol suspension and mandatory reporting to the federal funding agencies that support the Principal Investigator.**

Training and Personnel Requirements

Euthanasia must be carried out by personnel properly trained in the procedure being used. This is especially important when physical methods such as decapitation, cervical dislocation or pithing are used, since these

methods require a certain amount of expertise to assure a humane outcome. It is the PI's responsibility to assure that all persons performing euthanasia are properly trained and supervised. **All individuals performing euthanasia as part of a research project must be listed on the approved protocol.**

The clinical staff of the Animal Resources Center (ARC) is available to demonstrate and/or discuss euthanasia techniques.

Verification of Death

Proper euthanasia technique will include a physical examination or close observation to assure that the animal is dead prior to disposal. Death should be confirmed by personnel who can recognize cessation of vital signs in the species being euthanized. Whenever possible, the best method is to confirm the absence of a heartbeat, which is a reliable indicator of death in most species. Monitoring respiration by observing chest movement is less valuable, because a heartbeat may continue after visible respiration has ceased. If respiratory movement is the only criteria, observation should continue for a prolonged period after euthanasia (e.g., 10-15 minutes for mammals). Verification of death is especially important when CO_2 or anesthetic gases are used and the animal is discarded intact, i.e., it is not used for tissue harvest or other invasive postmortem procedures.

It is recommended that a secondary physical method such as decapitation, cervical dislocation or thoracotomy be used to assure death in birds and mammals. Pithing or rapid freezing are additional techniques that can be used to verify death of cold-blooded animals. A written description of the secondary physical method(s) to be used will be required for studies using gaseous anesthetics or CO_2 for euthanasia if animals are discarded intact.

Equipment Used for Physical Methods

Physical methods may include the use of instruments that are blunt (e.g., cervical dislocation), or sharp (e.g., decapitation or pithing). The Principal Investigator must assure that the choice of instrument is appropriate for the size and the anatomical conformation of the animal involved, with input from the Attending Veterinarian as needed. In many cases the use of specialized equipment such as a custom guillotine or enterotomy scissors will perform better than conventional scissors, knives or scalpels. Each lab must provide for the proper periodic evaluation and sharpening or replacement of equipment to assure proper function.

Best Practice Information

The primary source document for appropriate euthanasia practices is the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, last updated in 2007. However, the committee writing that report recognized that it cannot be considered an all-encompassing document, and the language allows the use of professional judgment based on other current literature sources. The following reference list includes some of the most useful and readily available sources to be used when euthanasia methods are being considered.

U.S. Guidance

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

- *Guide for the Care and Use of Laboratory Animals (1996)*
  Institute for Laboratory Animal Research
  http://fermat.nap.edu/books/0309053773/html

International Sources

- *Guide to the Care and Use of Experimental Animals – Euthanasia (1993)*
  Canadian Council on Animal Care
  http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/ GUIDES/ENGLISH/V1_93/CHAP/CHXII.HTM

- *Recommendations For Euthanasia Of Experimental Animals Part 1 (1996) and Part 2 (1997)*
  European Commission
  http://www.lal.org.uk/pdffiles/LA1.pdf http://www.lal.org.uk/pdffiles/LA2.pdf

- *Euthanasia of Animals Used for Scientific Purposes*
  Australian and New Zealand Council for the Care of Animals in Research and Teaching
  http://www.adelaide.edu.au/ANZCCART/ news/Euthanasia.pdf

Species-Specific Information

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

- *Guidelines For Use Of Live Amphibians And Reptiles In Field And Laboratory Research (2004)*
  American Society of Ichthyologists and Herpetologists
  http://www.asih.org/files/hacc-final.pdf

- *Guidelines To The Use Of Wild Birds In Research (1999)*
  THE ORNITHOLOGICAL COUNCIL
  http://www.nmnh.si.edu/BIRDNET/GuideToUse/Guidelines_2d_edition.pdf

- *Fish Research and the Institutional Animal Care and Use Committee (2003)*
  Institute for Laboratory Animal Resources
  http://dels.nas.edu/ilar_n/ilarjournal/44_4/v4404Borski.pdf

- *Guidelines for the Use of Fishes in Research (2004)*
  American Fisheries Society
  http://www.fisheries.org/afs/publicpolicy/guidelines2004.pdf

Study Considerations and Alternatives

It must be recognized that it is extremely important for experiments be planned and performed in a way that ensures the validity of the data produced. If the euthanasia method used interferes with the ultimate goals of the research study and makes the data unusable, then the lives of the animals may have been wasted. Careful consideration of the possible adverse effects of the various options available must occur. There may occasionally be special circumstances or situations in which options that are not listed in this document might be considered acceptable. These exceptions must be carefully considered by the investigator and the IACUC to assure the best outcome for the animals as well as the study.

Section 2 – Recommended Agents and Methods of Euthanasia Listed By Species

The selection of specific agents and methods for euthanasia will depend on the species involved and the objectives of

the protocol. Generally, inhalant or non-inhalant chemical agents (such as barbiturates, inhalant anesthetics, or CO_2) are preferable to physical methods (such as cervical dislocation or decapitation). However, scientific considerations might preclude the use of chemical agents for some experimental studies. All methods of euthanasia must be reviewed and approved by the IACUC. Specific justification will be required when physical methods are used as the sole method on fully conscious animals.

AMPHIBIANS

- Inhalant anesthetics
- Carbon dioxide (CO_2)
- Barbiturates
- Tricaine methane sulfonate (MS-222)
- Double pithing
- Benzocaine hydrochloride
- Chlorobutanol (Chlorotone)
- Physical methods such as decapitation or pithing after sedation
- Conditionally acceptable: Single pithing; decapitation; stunning followed by decapitation

BIRDS

- Inhalant anesthetics
- Carbon dioxide (CO_2)
- Barbiturates
- Physical methods such as decapitation or cervical dislocation after sedation
- Conditionally acceptable: cervical dislocation; decapitation

CATS/DOGS

- Inhalant anesthetics
- Carbon dioxide (CO_2)
- Barbiturates
- Potassium chloride or exsanguination (under general anesthesia)

FISH

- Tricaine methane sulfonate (MS-222)
- Benzocaine hydrochloride
- Quinaldine
- Clove oil
- Barbiturates
- Inhalant anesthetics
- 2-phenoxyethanol
- Chlorobutanol (Chlorotone)
- Physical methods such as decapitation followed by pithing after sedation
- Conditionally acceptable: stunning followed by decapitation/pithing; decapitation and pithing alone (smaller species)

NONHUMAN PRIMATES

- Barbiturates

- Potassium chloride or exsanguination (under general anesthesia)
- Conditionally acceptable: inhalant anesthetics; carbon dioxide (CO_2)

RABBITS

- Inhalant anesthetics
- Carbon dioxide (CO_2)
- Barbiturates
- Potassium chloride or exsanguination (under general anesthesia)
- Conditionally acceptable: cervical dislocation (< 1 kg); decapitation

REPTILES

- Barbiturates
- Inhalant anesthetics (in appropriate species)
- Carbon dioxide (CO_2) (in appropriate species)
- Physical methods such as decapitation after sedation
- Conditionally acceptable: stunning and decapitation; decapitation and pithing

RATS, MICE AND OTHER SMALL MAMMALS

- Inhalant anesthetics (halothane, isoflurane)
- Carbon dioxide (CO_2)
- Barbiturates
- Potassium chloride or exsanguination (under general anesthesia)
- Physical methods such as decapitation or cervical dislocation after sedation
- Conditionally acceptable: methoxyflurane; cervical dislocation (< 200 g); decapitation

SWINE

- Barbiturates
- Potassium chloride or exsanguination (under general anesthesia)
- Conditionally acceptable: inhalant anesthetics; carbon dioxide (CO_2)

Section C – Technical Comments on Agents and Methods

Inhalant Anesthetics

Because most inhalant anesthetics act as topical irritants in their liquid state, animals should be exposed to the vapors of the anesthetic only. Chambers must be designed to assure the animals don't come into contact with the wicking material that may be saturated with the liquid phase of the anesthetic. Sufficient air or oxygen must be provided during the induction period to avoid hypoxia prior to unconsciousness. All agents are given "to effect" until respiratory and cardiac arrest occurs.

Halothane and **isoflurane** have the most rapid action, and since halothane is better tolerated, it is preferred. Methoxyflurane is less suitable, due to its slow effect and poor market availability. Care should be taken to minimize personnel exposure to vapors.

Ether has historically been used as a euthanasia agent. However, it is highly flammable, can form explosive peroxides after exposure to air and light, and is known to be a distressful irritant when administered to animals. Considering the disadvantages, **ether should not be used for routine euthanasia** of laboratory animals. If an

investigator has a very compelling requirement for ether based on the needs of a particular study, a proposal can be submitted for consideration by the IACUC and the Office of Environmental Health & Safety (EH&S) to determine if there is sufficient justification and to assure that proper safety precautions will be taken.

Non-Anesthetic Gases (NOTE: Most agents in this category require the use of special equipment.)

Carbon dioxide has long been the preferred technique for euthanizing rodents and other small laboratory animals. Use of a sealed chamber filled by a compressed gas cylinder is required. CO_2 generated by other methods, e.g., dry ice, is unacceptable because gas flow can't be regulated precisely. Chambers must not be overcrowded to avoid distress during the procedure. Because CO_2 can act as a reversible anesthetic, it is imperative that the animals be kept in the chamber for several minutes after respiratory arrest. In order to assure death after CO_2 in those circumstances where the animal is discarded intact (i.e., it is not used for tissue harvest or other invasive postmortem procedures), a physical means to assure death MUST be performed after CO_2 exposure. Examples of acceptable physical methods include cervical dislocation (for mice or rats no larger than 200 grams), decapitation or thoracotomy (making a stab incision into the chest to open up the lung cavity)

Due to physiologic characteristics, neonates require prolonged exposure to the gas. For more detailed information, refer to IACUC guidance document "Guidelines for the Use of Carbon Dioxide (CO_2) for Rodent Euthanasia."

Nitrogen, argon or **carbon monoxide** may be acceptable under specific and unique situations but have no clear advantages and are rarely if ever used in biomedical research.

Pharmacological Agents

Use of these agents requires adequate restraint and mastery of appropriate injection techniques. Barbiturates are acceptable for all species, but are most commonly used for mammalian species and birds. These drugs should be administered intravenously (IV) whenever possible, but intraperitoneal (IP) or intracoelomic administration is acceptable for rodents, amphibians, reptiles and fish. Intracardiac injection is an alternative, but this should be done only on animals that are sedated or anesthetized. **Sodium pentobarbital** is the most common barbiturate agent for euthanasia, used either alone or in commercially available euthanasia mixtures. The dosage is usually at least twice that required for anesthesia, and ranges from 85 mg/kg for larger species to 200 mg/kg for some rodents. A dosage of 120 mg/kg is sufficient for most species, but more should be given if death does not ensue. Commercial euthanasia formulations should be used following label directions (e.g., 1 ml/lb for Beuthanasia-D). Sodium pentobarbital is a Class II controlled substance that is tightly regulated. Investigators using this agent must have current federal (DEA) and state (DPS) registration approval and are required to store the drug in a locked location and maintain detailed daily use records. For more information, contact the Office of Environmental Health and Safety (EHS). Euthanasia using **potassium chloride** is permissible only in an anesthetized animal. Concentrated KCl should be given rapidly IV until rising serum potassium levels result in cardiac arrest.

Tricaine methane sulfonate (MS-222) is a useful agent for aquatic species. It can be used either as an injectable agent (200-300 mg/kg of a 1% solution in physiologic saline) or more commonly as an immersion bath (500 mg/liter in H_2O) for amphibians and fish. When used in freshwater, the pH of the solution should be tested and buffered to neutrality as needed with sodium bicarbonate. The immersion time needed to assure death can range from 20 minutes to three hours, so it may be advantageous to use MS-222 as an initial anesthetic step followed by a physical method of euthanasia. Note: Cutaneous exposure to MS-222 can cause retinal toxicity. Gloves should be worn at all times when handling fish and amphibians, and in particular when using MS-222. **Benzocaine hydrochloride** (250-500 mg/liter) can be used as an alternative for amphibians and fish.

Other useful immersion agents for fish include **quinaldine** (100 mg/liter) and **chlorobutanol** (300 mg/liter). **Clove oil** contains eugenol as the active compound, and the current literature states that it is an acceptable agent for fish euthanasia (400 mg/liter). **2-phenoxyethanol** can be used (0.6 ml of the liquid compound per liter) but it is not a preferred method because it can be slow acting in some species and adverse reactions can occur prior to unconsciousness.

NOTE: The poor aqueous solubility of some of the agents used for immersion methods may require the initial preparation of a concentrated stock solution in an alternative solvent. Acceptable protocols for such preparation are available in the literature and should be used.

An overdose with non-barbiturate injectable anesthetic (e.g., ketamine/xylazine or tribromoethanol) is not acceptable as a sole method, but such drugs can be used to sedate or anesthetize animals prior to the use of a physical method in a two-step procedure.

Physical Methods (NOTE: These methods require that the user have experience and skill in the techniques to be used.)

Exsanguination is acceptable for all species if the animal is first rendered unconscious by another method. Rapid removal of blood can be accomplished by severing major vessels or (in smaller animals) by cardiac venipuncture.

Cervical dislocation is acceptable for mice, birds, rats (< 200 gm) and rabbits (< 1 Kg), but proper technique is essential. It is therefore recommended that animals be first sedated with another agent (carbon dioxide, pentobarbital or halothane are suggested). Its use as a sole means of euthanasia requires scientific justification and IACUC approval. For more detailed information, refer to IACUC guidance document "Guidelines for the Use of Cervical Dislocation for Rodent Euthanasia."

Decapitation with proper equipment may be performed on small mammals or birds after the animal has been sedated or lightly anesthetized (carbon dioxide, pentobarbital or halothane are suggested). Decapitation of fish, amphibians and reptiles should be followed by **cranial pithing** (see below) to assure rapid loss of brain function. Use of decapitation as a sole means of euthanasia in any species requires scientific justification and IACUC approval. Decapitation should generally be used only when study design requires it due to the potential hazard to personnel and the possibility of operator error leading to a prolonged or distressful death. Many species react adversely to the smell of blood, so animals should not be decapitated in the presence of other animals and the person performing decapitation should change gloves and/or wash hands between animals. Adult rodents should be decapitated with a commercially available guillotine unless the procedure is performed under anesthesia or after cervical dislocation.

Pithing is the insertion of an instrument into the central nervous system (brain case or spinal canal) to quickly disrupt consciousness and cause death. Pithing of both the brain and the spinal cord (double pithing) may be used as the sole means of euthanasia in frogs of the genus *Rana* or other amphibians with anatomic features that facilitate easy access to the central nervous system. In all other amphibian and reptile species, pithing should be followed by complete decapitation.

Other physical methods - Under very specialized circumstances, other methods such as stunning, thoracic compression or air embolism (under anesthesia) may be allowed in small species if research needs make it necessary and there are no available alternatives. Use of hypothermia (ice water) or freezing is not considered to be acceptable for routine euthanasia, although hypothermia can be used as an adjunct method in fish, amphibians or reptiles prior to the use of a physical method. Freezing can be used as a secondary method to assure death after a chemical or physical method has been used to kill the animal (often useful for ectotherms) but, if an anesthetic gas or CO_2 has been used, another physical method should be used to assure death prior to placing animals in a freezer.