Euthanasia Guidelines

Animals are normally euthanized at the end of a study for the purpose of sample collection or post-mortem examination. Animals may be euthanized because they are experiencing pain or distress. Euthanasia is defined as a pain-free or stress-free death. The IACUC has approved certain methods for humanely killing animals that meet the definition of euthanasia. The appropriateness of the method may vary from species to species. These guidelines are adapted from the report of the Americal Veterinary Medical Association Panel on Euthanasia, J Am Vet Med Assoc 2007. The American College of Laboratory Medicine has issued a report on rodent euthanasia that has many useful references and guidelines.

Investigators or technicians who require advice or assistance on proper techniques of euthanasia may contact RAR.
NOTE: You may only use a euthanasia method that is approved in your IACUC Animal Care and Use Protocol. A change in euthanasia method, including dose or route of administration, is a significant change in protocol and must be reviewed and approved by the IACUC before implementation.

CRITERIA FOR EUTHANASIA

Euthanasia of animals is expected if animals demonstrate the conditions listed below, whether the animal has been manipulated or not. Additional criteria may be specified on the Animal Usage Form. Fulfillment of one criterion can constitute grounds for euthanasia. **Exceptions** are permitted only if approved by the IACUC as part of the protocol review process (i.e. the clinical signs listed below are expected as part of the experiment and appropriate measures are taken to minimize pain or discomfort in the animals).

1. **Weight loss**: loss of 20-25% (depending on attitude, weight recorded at time of arrival, and age: growing animals may not lose weight, but may not gain normally) or if not measured, characterized by cachexia and muscle wasting.
2. **Inappetance**: complete anorexia for 24 hours in small rodents, up to 5 days in large animals; partial anorexia (less than 50% of caloric requirement) for 3 days in small rodents, 7 days in large animals.
3. **Weakness/inability to obtain feed or water**: Inability or extreme reluctance to stand which persists for 24 hours, assuming that the animal has recovered from anesthesia.
4. **Moribund state**: In rodents, measured by a lack of sustained purposeful response to gentle stimuli (example of purposeful response- weak attempt to get up; if animal is on its side, attempts should be asymmetrical in nature); in larger animals, measured by depression coupled with body temperature below 99°F (assuming in either case that the animal has recovered from anesthesia).
5. **Infection**: infection involving any organ system (either overt, or indicated by increased body temperature or WBC parameters) which fails to respond to antibiotic therapy within an appropriate time and is accompanied by systemic signs of illness.
6. **Signs of severe organ system dysfunction non-responsive to treatment, or with a poor prognosis as determined by an RAR veterinarian**:
   Respiratory: dyspnea, cyanosis.
   Cardiovascular: blood loss or anemia resulting in hematocrit below 20%; one transfusion

may be performed.
Gastrointestinal: severe vomiting or diarrhea, obstruction, intussuception; peritonitis, evisceration (immediate euthanasia required).
Urogenital: renal failure characterized by elevated BUN, creatinine or uroperitoneum.
Nervous: CNS depression, seizures, paralysis of one or more extremities; pain unresponsive to analgesic therapy.
Musculoskeletal: muscle damage, bone injury, locomotor defecits, etc. resulting in inability to use the limb, unless anticipated as part of the study.
Integumentary: Non-healing wounds, repeated self-trauma, second or third degree heating pad burns.

SURGERY TO CORRECT EXPERIMENTAL COMPLICATIONS

Only one major surgical procedure (involving entry of abdomen or thorax) may be performed per animal, unless indicated on an approved protocol. Therefore, major surgery intended to correct complications arising after a major experimental procedure is not permitted without prior approval. In such cases, euthanasia must be performed. Procedures such as repair of dehiscences and wound cleaning/debridement for treatment of infection may be performed following notification of the RAR veterinary staff.

Method	Animals under 125 g	Rabbits/Rodents over 125 g under 1 kg	Rabbits/Rodents over 1 kg under 5 kg	Amphibians[3]/Fish
CO_2	A	A[4]	A[4]	A
Barbiturate Overdose/ Euthanasia Solution, Intraveneous (100 mg/kg)	A	A	A	A
Barbiturate Overdose/ Euthanasia Solution, Intraperitoneal (100 mg/kg)	A	A	A	A
Anesthesia and Exsanguination	A	A	A	A
Anesthesia and Intraveneous KCl (1-2 meq/kg)	A	A	A	A
Anesthesia and Decapitation	A	A	AWJ	A
Anesthesia and Cervical Dislocation	A	A	UNA	N/A
Decapitation of Awake Animal	AWJ[1]	AWJ	AWJ	AWJ
Cervical Dislocation of Awake Animal	AWJ[2]	UNA	UNA	N/A
Other				A- Immersion in MS-222 (tricaine) or benzocaine at 2 g/L water

Acceptable Methods for Euthanasia of Animals RAR formulary dosages.

Volatile agents used to euthanize animals should not be stored or used in animal rooms because of improper ventilation, toxicity to laboratory animals, and possible effects on experimental results.

Chloroform is not acceptable for either anesthesia or euthanasia as it is very toxic to many species of mice. Additionally, this compound has been shown to be carcinogenic.

Ether is irritating, flammable and explosive, and should not be used in animal rooms. In addition, animals euthanized with ether must be left in a fume hood for several hours so that the carcasses

are not explosive when disposed of. Precautions on ether use are available from DEHS.

Chloral hydrate and alpha chloralose used as sole agents are not adequate to reliably achieve euthanasia

Abbreviations:

- A = Acceptable
- AWJ = Acceptible only with scientific justification, in writing, on the Animal Usage Form, that another methods would interfere with the goals of the experiment
- UNA = Unacceptable
- N/A = Not applicable or not specifically addressed by the IACUC
- **Always UNACCEPTABLE** in awake animals: KCl, MgSO4, strychnine, neuromuscular blocking agents, exsanquination, air embolism, freezing and chloroform (due to its hazards to personnel).

[1 + 2] Unless precluded by scientific considerations, it is required that all animals be sedated or anesthetized before decapitation or cervical dislocation
[3] Amphibians may also be double-pithed
[4] It is recommended that rabbits not be euthanized by CO_2 inhalation because of difficult induction.
[5] Swine <40 kg may be euthanized with CO2 in an appropriate chamber.
[6] Neonatal swine may be euthanized by IP barbiturate injection.

Standard Euthanasia Methods for Commonly Used Species

Below are a set of standard acceptable euthanasia methods. You may cut and paste them into the Animal Care and Use Protocol (ACUP) form, section 6C.1). Please contact the IACUC or RAR veterinary staff if you have any questions about these methods or would like training in the use of these methods.

Rodents (Mice, Rats, Gerbils, Hamsters, Guinea Pigs, and Voles)

- Carbon dioxide (CO2) To effect
- Sodium Pentobarbital 100 or > mg/kg IV, IP
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) (e.g. Beuthanasia®, Euthasol®, Fatal-Plus®, Somlethal®) 0.22 ml/kg IV, IP (~86 mg/kg pentobarbital)
- Decapitation under anesthesia (anesthesia details must be specified in ACUP)
- Cervical dislocation under anesthesia (anesthesia details must be specified in ACUP)

Rabbits

- Sodium Pentobarbital 100 or > mg/kg IV, IP
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) (e.g. Beuthanasia®, Euthasol®, Fatal-Plus®, Somlethal®) 0.22 ml/kg IV, IP (~86 mg/kg pentobarbital)
- Exsanguination under anesthesia (anesthesia details must be specified in ACUP)

Amphibians and Fish

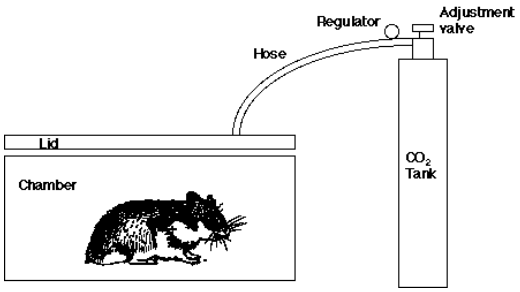
- Sodium Pentobarbital 100 or > mg/kg IV, ICL
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) (e.g. Beuthanasia®, Euthasol®, Fatal-Plus®, Somlethal®) 0.22 ml/kg IV, ICL (~86 mg/kg pentobarbital)
- Benzocaine hydrochloride 250 mg/liter (Water bath)
- Tricaine methane sulfonate (e.g. MS-222®) 3 g/liter (Water bath)

IC = intracardiac
ICL = intracoelomic
IP = intraperitoneal
IV = intravenous

USE OF THE CO_2 CHAMBER FOR EUTHANASIA OF RODENTS

DIRECTIONS

1. Whenever possible, **euthanize animals in their home cage** rather than transferring them to a new cage or chamber for euthanasia.
2. Do **not** pre-fill the cage or chamber with CO_2.
3. Open the tank and adjust the regulator to read:
- no higher than 5 psi or
- 1 liter/minute for small cage (mouse box)
- 4.5 liters/minute for large cage (rat box)
4. **Fill slowly** to minimize nasal/occular irritation & aversion to CO_2.
5. Wait approximately 3-5 minutes for animal to stop moving or breathing. Eyes should be fixed and dilated.



VERIFICATION OF COMPLETE EUTHANASIA IS MANDATORY. THE ANIMAL IS NOT DEAD IF:

1. Its heart is beating, check this by feeling the chest between your thumb and forefinger.
2. It blinks when you touch the eyeball.
3. If the animal is not dead, place it back in the chamber, recharge and wait another 5 minutes or, use scissors to open the chest cavity and create a pneumothorax. MAKE SURE THE ANIMAL IS NOT AWAKE WHEN YOU DO THIS!