Iowa State University  
Institutional Animal Care and Use Committee

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Approved Date: 5-17-2010</td>
<td>Policy Title: Euthanasia</td>
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<td>Last Reviewed: 9-5-2018</td>
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</tbody>
</table>

Institutional Animal Care and Use Committee (IACUC) Policy

EUTHANASIA

1. Background

The National Research Council Guide for the Care and Use of Laboratory Animals states that methods of euthanasia must “induce rapid unconsciousness and death without pain or distress.” Iowa State University observes the above-stated definition and follows the euthanasia guidelines specified in the American Veterinary Medical Association Guidelines on Euthanasia (AVMA, 2013). The purpose of these guidelines is to describe acceptable methods for the euthanasia of animals used in teaching, research and testing at Iowa State University.

2. Policy

Review of Euthanasia Methods by the IACUC

Euthanasia techniques and/or methods must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). Research and teaching protocols must include the method of euthanasia that will be utilized when required by the protocol and must also include the methods that will be used to minimize and relieve unalleviated pain and distress. The protocol must specify criteria that will be used to determine when euthanasia may be required, so that the investigator and/or veterinarian can ensure humane endpoints and fulfillment of protocol objectives.

The IACUC uses the AVMA Guidelines on Euthanasia for all its recommendations. Investigators and instructors may only use a euthanasia method that is approved in the IACUC protocol. A change in euthanasia method, including dose or route of administration, may be reviewed by veterinary verification and consultation (VVC) process if the change is to another AVMA approved method.
Only trained personnel listed in the IACUC approved protocol may perform euthanasia. The principal investigator is responsible for ensuring that personnel performing euthanasia have received training to perform the procedure used. Training in euthanasia procedures is available from LAR veterinarians and staff. In the event that an individual listed in the protocol cannot be contacted in a timely matter in a medical emergency, the decision to treat or euthanize an animal will be made by the Attending Veterinarian (AV) or a Laboratory Animal Resource (LAR) clinician.

Death must be verified by the assurance of the cessation of respiratory and cardiovascular movements by observation at room air for at least 10 minutes, or by employing a secondary method of euthanasia such as cervical dislocation, decapitation, or bilateral thoracotomy prior to carcass disposal.

In field settings, decisions about euthanasia most often arise because of difficulties associated with capture, handling, and other investigator-caused circumstances. The IACUC protocol must identify criteria when humane euthanasia will be the expected outcome.

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 IACUC protocol review 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, body condition score (see Figure 1), and age: growing animals may not lose weight, but may not gain normally) or if not measured, characterized by cachexia and muscle wasting.
2. **Body condition score (BCS)**: BCS less than or equal to 2 in addition to weight loss, decreased appetite, or other signs of chronic disease.
3. **Inappetence**: 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.
4. **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.
5. **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).
6. **Infection**: Infection involving any organ system (either overt or indicated by increased body temperature or WBC parameters) that fails to respond to antibiotic therapy within an appropriate time and is accompanied by systemic signs of illness.

7. **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, intussusception; 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 deficits, 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.
Acceptable Methods for Euthanasia of Animals
The following is a table of IACUC-approved methods for humanely euthanizing animals in laboratory settings. The guidelines in the table are adapted from the AVMA Guidelines for Euthanasia (2013).

<table>
<thead>
<tr>
<th>Species</th>
<th>Acceptable</th>
<th>Acceptable with conditions</th>
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<tbody>
<tr>
<td>Amphibians</td>
<td>As appropriate by species: injectable barbiturates, dissociative agents and anesthetics, topical buffered MS222, benzocaine hydrochloride</td>
<td>As appropriate by species: Inhaled anesthetics (see specifications), CO2, blunt force trauma to head, rapid freezing</td>
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<tr>
<td>Birds</td>
<td>Intravenous barbiturates</td>
<td>Inhaled anesthetics, CO2, cervical dislocation (small birds and poultry), decapitation (small birds)</td>
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<tr>
<td>Fish</td>
<td>Immersion in buffered benzocaine or benzocaine hydrochloride, isoflurane, buffered MS222, injected pentobarbital, rapid chilling (zebrafish)</td>
<td>Eugenol, isoeugenol, clove oil, decapitation/cervical transection/blunt force trauma followed by pithing</td>
</tr>
<tr>
<td>Horses</td>
<td>IV Barbiturates</td>
<td></td>
</tr>
<tr>
<td>Rabbits</td>
<td>Intravenous or intraperitoneal barbiturates</td>
<td>Inhaled anesthetic overdose, cervical dislocation (&lt;1kg), penetrating captive bolt</td>
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<tr>
<td>Reptiles</td>
<td>As appropriate by species: Injected barbiturates, dissociative agents and anesthetics as specified</td>
<td>As appropriate by species: inhaled anesthetics as specified, CO2, blunt force trauma to the head, rapid freezing &lt;4g</td>
</tr>
<tr>
<td>Rodents</td>
<td>Injected barbiturates and dissociative agent combinations</td>
<td>Inhaled anesthetics, CO2, cervical dislocation, decapitation</td>
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<tr>
<td>Ruminants</td>
<td>IV Barbiturates</td>
<td>Penetrating captive bolt</td>
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<tr>
<td>Swine</td>
<td>IV barbiturates</td>
<td>&lt; 12 pounds – CO2, blow to head (&lt;3 weeks) &lt;70 pounds- CO2, non-penetrating captive bolt &gt;70 to adult- penetrating captive bolt</td>
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<tr>
<td>Free Ranging Wildlife</td>
<td>Two stage method is preferred. General anesthesia or deep sedation followed by barbiturate overdose.</td>
<td>CO2, gunshot</td>
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Please consult with a LAR veterinarian for further information or for information on euthanasia in specific species.

Guidelines to the Use of Wild Birds in Research
(https://www.aaalac.org/accreditation/RefResources/SS_WildBirds.pdf)

Thoracic compression in small birds and mammals- AAALAC recognizes that in certain situations traditional euthanasia techniques are not feasible. The IACUC has authority to approve this method based on protocol justification, appropriate training for the technique is provided, and continued approval is re-evaluated as more scientifically based data regarding its use becomes available.

Guidelines for Euthanasia of Fetal and Neonatal Rodents

1. Fetus to birth: If required for study, individual euthanasia of fetuses can be done by decapitations with surgical scissors or cervical dislocation. Intraplacental injection of phenobarbital may be justified for procedures which require preservation of tissues. If fetuses not required for study then pregnant dam can be euthanized following above guidelines listed above for adult rodent euthanasia.

2. Neonates up to 10 days of age: Acceptable methods include phenobarbital injection, decapitation, or cervical dislocation. Inhalant anesthetics (CO2 or Isoflurane) require a secondary method of euthanasia (cervical dislocation, decapitation, bilateral pneumothorax).


5. Hypothermia: acceptable method for fetuses and altricial neonates up to 7 days of age (avoid direct contact with ice/cold surface).

6. Liquid nitrogen freezing: can be used at <5 days in altricial neonates and unconscious fetuses. For neonates 5 days or greater can be used if preceded by anesthesia.

3. References and Regulations


Direct questions about this document to: Institutional Animal Care and Use Committee, Iowa State University, 515-294-1516, iacuc@iastate.edu
Appendix I. Isoflurane Emergency Euthanasia Field Kit

*Keep in stock:*

**Isoflurane**

**Gauze or cotton balls**

**Anesthetic induction chamber**—This should be a sealable container that is an appropriate size for the species you work with. The container should also be clear walled in order to monitor the animal during the anesthesia process. Examples include a gallon size plastic zip top bag for animals that are mouse size and smaller, or a large glass jar or plastic storage tub with lid. In the example pictured to the right the induction chamber is used as a container for the rest of the kit. To reduce isoflurane exposure, anesthesia work should be done outside.

**Log book**—Keep a record of the occasions the emergency euthanasia kit is used. Note the date, what was euthanized, why it was euthanized, any secondary method used (cervical dislocation, etc.), and who performed the euthanasia.

**Waste containment**—a small plastic bag or 45 ml conical tube to hold used isoflurane-soaked cotton or gauze until it can be disposed of properly

**Gloves**—for handling sick and injured animals and their carcasses

**Goggles and mask**—for personal protection from injury or zoonotic disease when handling sick wildlife

**Contacts:**

- In case of injury to personnel (e.g., bites or scratches from wildlife):
  - McFarland Occupational Medicine
  - Phone: 515-239-4496

- In case of adverse events involving animal health and welfare:
  - Dr. Mary Sauer, Attending Veterinarian
  - Phone: 515-509-7264