

IACUC Guidelines for Euthanasia of Rodents

General Considerations:

- **Methods of Euthanasia must be consistent with the 2020 Edition of the AVMA Guidelines for Euthanasia of Animals**
- **It is imperative that death be verified after euthanasia and before disposal of the animal.**
- **Only trained personnel should perform euthanasia.**
- **Physical methods of euthanasia require demonstration of competence and may be approved with scientific justification in the IACUC protocol.**

Standard Euthanasia Methods for Commonly Used Species (Note: Alternative methods may be used if categorized as an approved method in the AVMA Guidelines or with a scientific justification and IACUC approval)

Rodents weighing > 500grams

Acceptable Methods of Euthanasia

- Carbon dioxide according to UWM SOP
- Overdose with isoflurane (see “Isoflurane Euthanasia” below)
- Sodium Pentobarbital > or = to 100 mg/kg IV or 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 or cervical dislocation of anesthetized animals
- Cervical dislocation of conscious mice by individuals that have demonstrated a high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized

Methods of Confirmation of Euthanasia

- Bilateral thoracotomy
- Decapitation
- Vital tissue harvest (inclusive of heart and/or lungs and/or brain)

Rodents weighing <500 grams**Adults and neonates > 10 days of age****Acceptable Methods of Euthanasia**

- Overdose of chemical anesthetics (2-3 times the anesthetic dose)
- Overdose of isoflurane (see “Isoflurane Euthanasia” below)
- CO₂ exposure according to UWM SOP
- Barbiturate overdose

Methods of Confirmation of Euthanasia

- Cervical dislocation (not acceptable for rats > 200 grams of body weight)
- Decapitation
- Bilateral thoracotomy
- Vital tissue harvest (inclusive of heart and/or lungs and/or brain)

Mouse and Rat Neonates < 10 days of age**Acceptable Methods of Euthanasia**

- Overdose of chemical anesthetics (2-3 times the anesthetic dose)
- Decapitation
 - Per NIH guidelines, decapitation alone for this age group is an acceptable means of euthanasia.

Methods of Confirmation of Euthanasia

- Decapitation

Mouse and Rat Feti 15 days of gestation to birth**Acceptable methods of Euthanasia**

- Decapitation with scissors or cervical dislocation
- Confirmed euthanasia of mother (feti not required for study)
- Confirmed euthanasia of mother (feti required for study)
 - The uterus with the pups or the pups with the amniotic can be removed after euthanasia of the mother
 - If at any point a fetus is allowed to breathe it must be decapitated

- Rapid freezing of feti while anesthetized (liquid nitrogen immersion)
 - Anesthesia may be effectively induced by hypothermia of the fetus, which can be achieved by submerging the fetus (with the amniotic sac intact) in cold (4-8⁰C/35-39⁰F) physiological saline until the fetus becomes completely immobile
 - If at any point the fetus is allowed to breath it must be decapitated

Methods of confirmation of euthanasia

- No further method of confirmation of euthanasia of the feti required

Mouse and Rat Feti up to 15 days' gestation

Acceptable Methods of Euthanasia

- Confirmed euthanasia of mother
 - Removal of feti from the anesthetized mother
 - No further method of confirmation of euthanasia of the feti required

Euthanasia by Perfusion-Rodents

- Any animal undergoing perfusion must be under a surgical plane of anesthesia before any incisions are made. A surgical plane of anesthesia must be maintained until the heart stops.

Isoflurane Euthanasia

- Adjust the isoflurane flow rate or concentration to 5% or greater
- Continue isoflurane exposure until one minute after breathing stops

Abbreviations

IC = intracardiac

ICL = intracoelomic

IP = intraperitoneal

IV = intravenous