

Methods of Euthanasia

General Recommendations for Euthanizing Laboratory Animals

The NIH ARAC has developed useful guidelines regarding rodent euthanasia using carbon dioxide (http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Adult.pdf) and special guidance for rodent neonates and feti in the intramural research program (http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Pup.pdf). The CCR ACUC supports this guidance and has developed the following guidelines that are relevant to NCI's animal program.

LASP is available to determine the appropriate flow rates for euthanasia chambers and to optimize humane euthanasia practices with laboratory-specific instructions and training. Confirmation of death and proper carcass disposal are essential. Carcasses and body parts are discarded as MPW in accordance with NIH DOHS policies on waste disposal.

Approved Methods for Euthanizing Laboratory Animals

Species	Age	Approved Methods	Approved With Justification
Mice, Rats	>7 days	<ol style="list-style-type: none"> 1) Carbon dioxide overdose 2) Injection of an overdose of anesthetics (barbiturates or dissociatives) 3) Anesthetic Gas overdose 	<ol style="list-style-type: none"> 1) Cervical dislocation 2) Decapitation 3) Microwave beam irradiation
Mice, Rats	≤7 days	<ol style="list-style-type: none"> 1) Injection of an overdose of anesthetics (barbiturates or dissociatives) 2) Decapitation 3) Cervical Dislocation 4) CO₂ or isoflurane overdose with prolonged exposure and secondary physical method 	<ol style="list-style-type: none"> 1) Rapid freezing in liquid nitrogen (if <5 days of age) with prior anesthesia
Mice, rats	Fetus	<ol style="list-style-type: none"> 1) Euthanasia of dam will result in euthanasia of feti 2) If euthanasia of individual feti required <ol style="list-style-type: none"> a) Decapitation b) Cervical dislocation 	<ol style="list-style-type: none"> 1) Rapid freezing in liquid nitrogen (if <5 days of age) with prior anesthesia
Nonhuman primates	Any age	<ol style="list-style-type: none"> 1) Overdose of pentobarbital +/- phenytoin (euthanasia solution) 	<ol style="list-style-type: none"> 1) Saline or fixative perfusion
Zebrafish	>3 days post fertilization (dpf)	<ol style="list-style-type: none"> 1) Rapid chilling (10 minutes adults, 20 minutes fry) 2) Immersion in anesthetic solution (for example buffered MS222 or buffered benzocaine) 	<ol style="list-style-type: none"> 1) Decapitation 2) Cervical transection Maceration

Zebrafish	≤3 days post fertilization (dpf)	1) Rapid chilling or immersion in anesthetic + exposure to dilute bleach or other secondary method	
Xenopus	Any age	1) Physical method after anesthesia (MS222)	

Brief Comments Regarding Methods:

- 1) CO₂ overdose
 - a. Induction at 10-30%
 - b. Please see <http://oacu.od.nih.gov/ARAC/documents/Rodent Euthanasia Adult.pdf> and/or <https://ncifrederick.cancer.gov/Lasp/Sop/default.aspx> for details regarding this procedure
- 2) Inhaled anesthetics
 - a. May need to be exposed for prolonged periods
 - b. Maintain compatible groups, etc as for CO₂ euthanasia
 - c. Use Scavenging system and approved containers to avoid environmental contamination (i.e. downdraft table)
- 3) Cervical dislocation
 - a. Only mice and rats <200g.
 - b. Requires training/experience.
 - c. Requires scientific justification in the ASP if done without anesthesia and person performing must have demonstrated technical competency
 - d. Demonstration of training is the responsibility of the PI/lab staff
 - e. Motor activity is not uncommon.
 - f. The trained personnel should be named in the protocol. This is because an untrained person could do this improperly, which may not result in rapid death
- 4) Decapitation
 - a. Motor activity will occur.
 - b. Segregate this activity from other awake animals.
 - c. Training or experience and a dedicated guillotine device for adult rodents with a sharpened blade required.
 - d. Sharp scissors or blade are appropriate for neonates/feti.
 - e. Justification and approval in the ASP is required
- 5) Injectable anesthetic overdose
 - a. For drug doses and recommendations contact LASP veterinary staff
 - b. IV (preferred) or IP routes only
 - c. ~100 mg/kg IV or 120 mg/kg IP for rodents
 - d. ~100 mg/kg IV for NHPs
- 6) Microwave beam irradiation
 - a. Used to fix brain metabolites without disrupting anatomy
 - b. Required a dedicated focused microwave beam irradiator.
 - c. DO NOT use a household microwave
- 7) Rapid chilling
 - a. Neonatal mice
 - i. Immersion in liquid nitrogen is acceptable for altricial neonates <5d of age that cannot perceive pain because of insufficient nervous system development

- b. Zebrafish
 - i. Immersion in 2-4°C water for 10 minutes (adults) or 20 minutes (fry 4-7 dpf) beyond operculum movements is acceptable
 - ii. The water bath should be formed by ice water with a pool in the middle
- 8) MS222 overdose
 - a. In frogs, concentrations of 5g/L result in rapid anesthesia but euthanasia may take up to one hour. Higher concentrations should be used and a secondary physical method
 - b. In fish, concentrations of 10g/L can be used and buffered with NaHCO₃ to reach a pH of 7-7.5. Fish should be immersed for 10 minutes following loss of operculum movements.

References:

- 1) AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
(http://oacu.od.nih.gov/regs/AVMA_Euthanasia.pdf)