THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

STANDARD EUTHANASIA GUIDELINES FOR RODENTS
(revised May 2009)

General Considerations:

- Euthanasia techniques must be consistent with the AVMA Guidelines on Euthanasia, June 2007. Methods are chosen to minimize animal pain and distress consistent with needs of the research protocol.

- The method of euthanasia must be specified in the approved Institutional Animal Care and Use (IACUC) protocol. Any chemical euthanasia method must be followed by a physical method from which the animal cannot recover such as decapitation, cervical dislocation, bilateral thoracotomy, tissue perfusion, or dissecting of a major organ.

- Use of anesthetic for euthanasia must be an overdose, not an anesthetic dose. Regardless of amount of chemical administered, animal must be completely non-responsive to noxious stimuli (hind paw pinch) before any physical means are applied.

- Sodium pentobarbital and ketamine are controlled substances and must be maintained according to the UCSF Controlled Substances Program.

- Physical methods of euthanasia such as decapitation or cervical dislocation of unanesthetized animals require demonstration of competence, and may be approved with proper justification in the IACUC protocol.

- The techniques listed below are suggested common methods for euthanasia of rodents. Other methods outlined in the AVMA Panel on Euthanasia are acceptable with proper justification in the approved IACUC protocol.

Techniques:

*Mice > 8 days old*

1. Intraperitoneal injection of at least 200 mg/kg sodium pentobarbital euthanasia solution, or other anesthetic at an overdose. Pentobarbital is best diluted to a concentration of no more than 60 mg/ml. Intraperitoneal injection must be followed by cervical dislocation, decapitation, bilateral thoracotomy, or other physical means from which an animal could not recover, after the animal has been determined to be non-responsive to noxious stimuli.

2. Inhalation of CO₂ from a pressurized tank in an uncrowded cage. A standard size mouse cage may contain no more than 10 adult mice or 2 litters. Follow CO₂ with cervical dislocation, decapitation or bilateral thoracotomy. The regulator for the CO₂ tank should be turned no higher than 6 psi. Animals must be left in the chamber for a sufficient time (4 or 5 minutes) so that complete asphyxia has been attained. Whenever possible euthanize mice in their home cage to minimize the stress of
being handled and placed into an unfamiliar enclosure.

**Mice and Rats < 8 days old**

1. It is acceptable to place neonates in a CO$_2$ chamber for 4-5 minutes first to anesthetize them, then follow with decapitation. Alternatively, rapid decapitation with sharp scissors may be performed. Remove all other live mice from workspace when performing this procedure.

2. Intraperitoneal injection of at least 200 mg/kg sodium pentobarbital euthanasia solution. Pentobarbital should be diluted to a concentration of no more than 60 mg/ml. After testing for response to painful stimuli, follow with cervical dislocation, decapitation, bilateral thoracotomy, or other physical means from which an animal could not recover.

3. Inhalation of CO$_2$: Place neonates in an uncrowded cage. Place cage in chamber, and slowly fill with CO$_2$ for 4 or 5 minutes. Leave animals in the chamber for at least one half hour. After one half hour, test for response to painful stimuli, then place in a bag and freeze. Note: this procedure may not be done in facilities where a freezer (-10 degrees C) is not available.

**Rats and Hamsters**

1. Intraperitoneal injection of at least 200 mg/kg sodium pentobarbital euthanasia solution. This solution is viscous and is best diluted to a concentration of no more than 60 mg/ml. Intraperitoneal injection must be followed by cervical dislocation, decapitation, bilateral thoracotomy, or other physical means from which an animal could not recover after the animal has been determined to be non-responsive to noxious stimuli. Cervical dislocation may not be performed on animals weighing greater than 200 grams.

2. Inhalation of CO$_2$ from a pressurized tank in an uncrowded cage, followed by cervical dislocation, decapitation or bilateral thoracotomy. The regulator for the CO$_2$ tank should be turned no higher than 6 psi. This allows for CO$_2$ to enter the chamber slowly so that complete narcotization occurs prior to asphyxiation. Animals must be left in the chamber for a sufficient time (4 or 5 minutes) so that complete asphyxia has been attained. Whenever possible euthanize rats and hamsters in their home cage to minimize the stress of being handled and placed into an unfamiliar enclosure. Cervical dislocation may not be performed on animals weighing greater than 200 grams.

**Guinea pigs**

1. Inject at least 200 mg/kg sodium pentobarbital euthanasia solution intraperitoneally. This must be followed by bilateral thoracotomy or other physical means from which an animal could not recover after the animal has been determined to be non-responsive to noxious stimuli.

2. Inhalation of CO$_2$ from a pressurized tank in an uncrowded cage, followed by cervical dislocation, decapitation or bilateral thoracotomy. The regulator for the CO$_2$ tank should be turned no higher than 6 psi. This allows for CO$_2$ to enter the chamber slowly so that complete narcotization occurs prior to asphyxiation. Animals must be
left in the chamber for a sufficient time (4 or 5 minutes) so that complete asphyxia has been attained. Whenever possible euthanize guinea pigs in their home cage to minimize the stress of being handled and placed into an unfamiliar enclosure.

**Disposal:**

The UCSF Biosafety Committee requires that all animal carcasses, tissues or organs contaminated with infectious agents be discarded in biohazard bags. Due to the difficulty of determining the infective status of most carcasses, the UCSF policy is to treat all carcasses as infected and they must be put in red biohazard bags. The sealed bags must be stored in closed waterproof containers in designated cold rooms or freezers until removed by the animal waste management contractors. **Do not place the red bags in dirty cages being transported to the cage wash facility.** Contact the Laboratory Animal Research Center (LARC) at 476-2204 for any information regarding carcass disposal.

**Training:**

Only trained individuals may perform euthanasia. Training is provided in individual or group workshops through the IACUC Training and Compliance.