MASS GENERAL RESEARCH INSTITUTE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON EUTHANASIA

GENERAL POLICY

This policy provides guidance, and the most commonly used and approved methods, for performing euthanasia of laboratory animals used in research at Massachusetts General Hospital (MGH).

Euthanasia is the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress. All euthanasia procedures performed within MGH animal facilities must be consistent with the recommendations outlined in the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.

Euthanasia is performed as required by protocol study endpoints, to relieve pain/distress and suffering from experimental manipulations or spontaneous conditions, and as appropriate in other situations deemed necessary by a CCM veterinarian. Animals that are experiencing undue pain/distress must be euthanized humanely unless these conditions are required for scientific objectives that have been documented and justified in a study protocol and approved by the IACUC.

It is important for researchers to delineate criteria for euthanasia in the IACUC protocol, including measurable physiological parameters and observable signs indicative of pain and distress. Euthanasia methods must be indicated, including the trained staff who will be responsible for observations and euthanasia procedures. CCM veterinary services should be consulted if needed to demonstrate and/or discuss these techniques.

Distress vocalizations, fearful behavior, and release of pheromones by a frightened animal can all cause anxiety and apprehension in other animals. Therefore, in general, animals should not be euthanized in the animal housing room/area to minimize stress on the remaining animals. Exceptions may occur given scientific justification (e.g., to prevent spread of infectious disease), for emergency euthanasia when an animal cannot be readily moved, or situations

that do not induce stress in the remaining animals (e.g., complete microisolator caging systems for housing rodents and thus will not be exposed to the effects mentioned above).

Any animal found to be “near death” (moribund) must be immediately addressed. Rodents found moribund by CCM staff will be euthanized in accordance with CCM SOPs and the Rodent Health Concern Booklet. For species other than rodents, when an animal is found moribund, attempts will be made to contact the Principal Investigator (PI) or specified contact person for that study. If the PI or contact person is unavailable, an agreement cannot be reached, or a delay will result in further distress to the animal, the CCM veterinarian has full authority by the IACUC and the Attending Veterinarian (AV) to euthanize the animal. No animal should ever be left in a moribund state.

Regardless of the technique used, all animals must be evaluated following euthanasia to confirm that they are dead. This includes observing or palpating the absence of a heartbeat and respiration. For rodents, an approved secondary physical method such as bilateral thoracotomy, cervical dislocation, exsanguination, or decapitation can also be used to confirm death. Animals may have absence of respiration but still have a heartbeat and recover fully. For large animals, adjunct vital signs used to assess death include fixed and dilated pupils. Confirmation of death is required because most euthanasia agents may also induce deep anesthesia that the animal can recover from if not administered properly. Failure to ensure death of animals after euthanasia procedures (e.g., failed euthanasia with CO_2) is a noncompliant event that must be reported to the regulatory agencies overseeing laboratory animal research.

All personnel performing animal euthanasia must be trained, knowledgeable, and proficient in the chosen techniques and verification of death. PIs are responsible for ensuring that their staff are trained in the relevant euthanasia method. Trained research staff are encouraged to euthanize their own animals whenever possible. CCM staff can euthanize rodents for research staff as a service.

If there are any questions about euthanasia or if proper equipment/drugs cannot be found, the CCM facility veterinarian or the On-Call veterinarian (if after hours, weekends or holidays) should be contacted immediately.

Carcasses must be disposed of immediately after euthanasia has been confirmed. Carcasses should be placed in a carcass bag, then should be placed

in walk-in coolers, refrigerators, or chest freezers specified for this purpose in CCM animal facilities or designated area in the laboratory.

EUTHANASIA METHODOLOGY GUIDELINES:
(For doses, refer to the species-specific Insight form or consult with your facility veterinarian.)

Adult Animals

A. Carbon Dioxide (CO_2) Asphyxiation using Compressed Gas
   1. **Dry ice is not an acceptable source of CO_2**
   2. Carbon dioxide must be supplied in a precisely regulated manner and in a purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank.
   3. CO_2 euthanasia stations and portable units are located in the CCM animal facilities with specific instructions posted in these areas
   4. Prefilled chambers are unacceptable
   5. CO_2 delivery must be maintained for at least one minute after respiration ceases
      a. Rodents (mice, rats, hamsters, guinea pigs, gerbils), avian
         i. Use CO_2 flow rate that displaces 30-70% cage volume/minute.
         ii. Euthanasia in the home cage is urged whenever possible to minimize stress (e.g., when euthanizing some but not all mice in a cage, keep the mice to be euthanized in the home cage and move the remaining mice to a clean cage).
         iii. Rodents from different cages should not be combined.
         iv. Rodent cages must not be overcrowded (follow the IACUC Policy on Rodent Breeding and Cage Density)
      b. Rabbits < 2 kg
         i. Use a CO_2 flow rate that displaces 50-60% cage volume/minute
         ii. Sedation is strongly recommended prior to exposure to CO_2

B. Barbiturates/Sodium Pentobarbital/Pentobarbital Combinations (e.g., Euthasol)
   1. Categorized as DEA Schedule II and/or III Controlled Substances and must be stored in a double-locked cabinet.
   2. Dosages should be 2-3 times the appropriate anesthetic dose
   3. Routes of administration should ensure rapid circulation

a. Intraperitoneal: mice, rats, hamsters, guinea pigs. May administer IP in rabbits, sedation/anesthesia recommended.
b. Intravenous: rabbits, dogs, swine, small ruminants, nonhuman primates, avian
c. Intracoelomic: amphibians
d. Intracardiac (under general anesthesia only): rabbits, dogs, swine, small ruminants, nonhuman primates, avian, ferrets

C. Dissociative Agent Combinations
1. Overdose used in rodents, rabbits
2. Ketamine must be used in combination with an alpha-adrenergic agonist (e.g., ketamine/xylazine anesthetic overdose)
3. Routes of administration:
a. Intraperitoneal: mice, rats, hamsters, rabbits, guinea pigs
b. Intravenous: rabbits

D. Inhalant Anesthetic (such as isoflurane)
1. Use of precision vaporizer is required. Bell jars are prohibited.
2. Adequate ventilation and scavenging must be provided to ensure personnel safety
3. May be used as overdose in rodents and avian species or as general anesthetic followed by non-survival surgery or secondary physical method (e.g., exsanguination) in rodents, rabbits, nonhuman primates, small ruminants, swine, dogs

E. Cervical Dislocation
1. Can be used as a secondary physical method to ensure death in anesthetized rodents < 200 grams and small birds.
2. To use as primary euthanasia means in non-anesthetized animals (rodents < 200 grams, small birds):
a. Must be scientifically justified in IACUC protocol
b. Personnel should be trained on anesthetized and/or dead animals
c. Personnel must demonstrate technical proficiency
d. Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

F. Decapitation with Guillotine or Scissors
1. Can be used as a secondary physical method to ensure death in anesthetized rodents and small birds.

2. To use as primary euthanasia means in non-anesthetized animals (rodents, small birds):
    a. Must be scientifically justified in IACUC protocol
    b. Personnel should be trained on anesthetized and/or dead animals
    c. Personnel must demonstrate technical proficiency to whom?
    d. Those responsible for the use of this method must ensure that personnel performing decapitation have been properly trained and consistently apply it humanely and effectively.
    e. Guillotine or decapitation scissors must be in good condition, kept clean and with sharp blades. Guillotines should be maintained as recommended by the manufacturer.
    f. Use of restraint methods, such as decapicones, is recommended for either guillotine or scissors methods.

G. Adjunctive Methods (Used Only in Anesthetized Animals)
    1. It is unacceptable to perform these methods in a conscious animal
    2. Animal must demonstrate Stage III anesthesia (surgical plane of anesthesia defined as loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli) before performing.
    3. Rodents, rabbits, dogs, swine, small ruminants, nonhuman primates
        a. Exsanguination:
            i. Collection of large volumes of blood (≥ 50% of total blood volume)
        b. Potassium Chloride:
            i. Intravenous or intracardiac route of administration to induce cardiac arrest
        c. Other Methods: Chemical perfusion, bilateral thoracotomy (pneumothorax), removal of vital organs (non-survival surgery)

H. Tricaine methanesulfonate (MS 222)
    1. Used in amphibians and fish
    2. Appropriate safety precautions should be taken when working with powder or concentrate
    3. A 10 gram/liter stock solution can be made and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution
    4. Stock solution must be protected from light and refrigerated or frozen if possible. Labelling and storage should be performed in accordance with the IACUC Labeling Guidelines and Recommended Expiration Dates for Research Drugs and Agents

a. Amphibian dose: 5 grams/liter.
b. Fish dose: 0.5 grams/liter
   Note: For zebrafish, 30 minutes exposure time following loss of rhythmic opercular movement is required.

Fetuses (mice and rats):

Up to and including 14 days of gestation:	Because the neural development is minimal, pain perception in the fetus is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death due to loss of blood supply and non-viability at this stage of development.
15 days - birth:	Possibility of pain perception documented in fetuses of this age; perinates are not sensitive to inhalants therefore chemical anesthesia (administered i.p.) is recommended. Decapitation, cervical dislocation, or rapid freezing are acceptable but may be aesthetically unpleasant. For fetuses that require chemical fixation, anesthesia such as hypothermia or a deep anesthesia of the mother with pentobarbital (which crosses the placenta) should be done prior to immersion or perfusion.

Neonates (mice and rats):

Birth – 10 days old (mice): Birth – 7 days old (rats)	Acceptable methods include IP pentobarbital or dissociative anesthetic overdose, decapitation, or cervical dislocation. Neonates can be exposed to inhalant gases or CO_2, but exposure times are extremely lengthy (e.g., up to 50 minutes in CO_2 for mice) and a secondary physical method must

	be used to assure death after no response is found to painful stimuli. For neonates requiring chemical fixation, anesthesia is required and after loss to painful stimuli determined, then neonates may be chemically fixed or perfused. Neonates ≤ 6 days old may be anesthetized using hypothermia (gradual cooling to 4 °C), followed by a secondary physical method after loss of movement.
Older than 10 days (mice): Older than 7 days (rats):	Follow guidelines for adults.

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