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STANDARD OPERATING PROCEDURE NO. 5
EUTHANASIA OF RODENTS

Euthanasia is from the Greek language meaning "good death". It is our responsibility to treat the laboratory animals in our care with respect and to insure a humane death. It is imperative that all animals be assessed carefully to assure that they are dead before disposal. Animals must be placed in plastic bags and sealed before placing in the freezer. All euthanasia methods must be performed by personnel trained in the procedure. The following methods are approved by the American Veterinary Medical Association Guidelines on Euthanasia (June 2007):

- **Carbon dioxide gas (CO_2) chamber** - for use with animals over 10 days old. Located in N110E Necropsy/Surgery room. Carbon dioxide gas (CO_2) is heavier than air and will sink to the bottom of the cage. Animals will quickly lose consciousness and stop breathing when exposed to the gas.
    - Transfer animals in their home cage with filter lid to N110E by cart.
    - Use scotch tape to cover auto water hole if applicable.
    - Replace filter lid with the stainless steel lid with CO_2 gas line attached.
    - Open main valve to carbon dioxide cylinder.
    - Use red valve to open flow to cage and fill chamber with gas.
    - Open red valve slowly to regulate gas flow to the cage. Do not let gas enter the cage at too high a rate to avoid distressing the animals. Observe the animals carefully. Most will become unconscious within 30 seconds.
    - Continue gas flow until all animals are unconscious. Monitor breathing and continue gas flow until breathing has stopped. Close red valve to stop gas flow. Leave lid on for additional few minutes to assure animals are dead.
    - Remove lid and verify that animals are dead, check for signs of breathing, heart beat or palpebral reflex. Remove animals and place in plastic bag.
    - Animals euthanized with carbon dioxide are eligible for donation to the raptor rescue center as long as they have not been exposed to any other hazardous chemicals. Animals can be placed in the green container in the chest freezer in the cage storage area.
    - Close main valve and bleed out excess gas by opening the red valve. Close the red valve.

- **Cervical Dislocation** - only for use in rats weighing less than 200 grams and in mice.
    - It is preferable to have the animal unconscious either by inhalation of carbon dioxide gas or by injectable anesthetics but can be done in a conscious animal by a well trained individual.
    - Place animal on surface that it can grip securely.

- Hold the tail and place solid rod (such as a pencil) behind the ears.
- In a quick motion, press forward and down with the pencil and pull backwards with the tail. This motion separates the cervical vertebrae from the skull and causes a rapid loss of consciousness.
- This method may also be used to supplement exposure to carbon dioxide gas and assure that animals are dead.
- Animals euthanized by this method are eligible for donation to the raptor rescue organization as long as they have not been exposed to any other hazardous chemicals. Animals can be placed in the green container in the chest freezer in the cage storage area.

- **Anesthetic Overdose** - can be either injectable barbiturate anesthetic or inhalation anesthetic.
  - Use 3x the normal anesthetic dose for injectable drugs. Inject as normal and monitor animal for cessation of breathing and heartbeat. This method is acceptable for animals less than 10 days old.
  - If using the gas anesthesia machine, utilize the induction chamber and deliver an overdose. Monitor the animal for cessation of breathing and heartbeat. Do not use this method in animals under 10 days old.
  - Animals euthanized by this method are not eligible for donation to the raptor rescue organization and must be incinerated. Animals can be placed in the red container in the chest freezer in the cage storage area.

- **Decapitation** - is an effective method that allows animal tissues to be harvested without any adverse effects of chemicals. It must be performed with supervision of the Vivarium manager.
  - Although it is preferable to perform decapitation under anesthesia, the method can also be used without prior sedation as long as a proper restraint device such as a Decapicone™ is used. The Decapicone™ is a plastic sleeve that restrains and positions the animal properly and prevents harm to personnel.
  - Use the guillotine in the sink with a low flow of water. Position the animal's neck beneath the blade and be careful to keep fingers out of the way of the blade. Press down firmly on the handle to perform the decapitation.
  - Animals euthanized by this method are not eligible for donation to the raptor rescue organization and must be incinerated. Animals can be placed in the red container in the chest freezer in the cage storage area.

- **Trans-cardial Perfusion under heavy anesthetization** - this method allows the animal to be perfused with formalin to preserve the anatomical structure of the brain. It must be performed with a deep level of anesthesia by injectable means and with the supervision of the Vivarium manager or trained investigator.
  - The animal is injected either IV or IP with the anesthetic and monitored for depth of anesthesia plane. The animal should be non-responsive to deep pain stimulation such as foot pad pinch with forceps.
  - Follow the established protocol for using the Perfusion One™ apparatus.
  - Animals euthanized by this method are not eligible for donation to the raptor rescue organization and must be incinerated. Animals can be placed in the red container in the chest freezer in the cage storage area.