7. Rodent Physical euthanasia Guideline_Support Doc.03

7.1 Materials

- Guillotine with sharp blades
- Decapicone
- Scissors
- Anesthetics
- Syringes and needles

Anaesthesia is necessary prior to physical methods of euthanasia, unless scientifically justified, described in the animal research protocol and approved by the authorities.

7.2 Cervical dislocation:

7.2.1. Verify that withdrawal reflex is absent by pinching the forelimb toes
7.2.2. Perform the procedure on a flat surface or surface where the animal can grip (e.g., the wire bar of the cage grid) if not anesthetized
7.2.3. Place the thumb and index finger on either side of the neck or at the base of the skull, or alternatively, press a rod at the base of the skull.
7.2.4. With the other hand, grasp the base of the trail between the thumb and forefinger and pinch firmly. Slightly elevate the hind limbs, no more than 20-30 degrees.
7.2.5. Quickly and firmly pull from the base of the tail in the direction away from the head and neck with simultaneously driving thumbnail down behind the base of the skull with the other hand.
7.2.6. Confirm death by exsanguination

7.3 Decapitation:

7.3.1. Verify that withdrawal reflex is absent by pinching the forelimb toes
7.3.2. Adult rodents
7.3.2.1. Place the animal in a decapicone
7.3.2.2. Quickly separate the head from the body at the cervical level with a guilliotine.
7.3.3. Neonatal rodents <2weeks of age
7.3.3.1. . Quickly separate the head from the body at the cervical level with a sharp scissor

7.4 Exsanguination

7.4.1 **For cardiac puncture** insert a needle (approximately 23 gauge) at a 30° angle to the left of the junction formed by the sternal appendix and the last rib.
7.4.2 **For abdominal aorta puncture**, incise the abdomen and retract the viscera to expose the aorta. Insert a needle (approximately 23 gauge for rats, 25 gauge for mice) into the vessel.
7.4.3 Withdraw the maximal volume of blood (approximately 1 ml in mice, and 10 ml in rats).
7.4.3.1 If blood collection is not required, the abdominal aorta may be cut following the approach detailed in 1.3.2
7.4.4 Verify that the animal is dead before disposing of the carcass by making sure there is no respiratory movement for at least 3 minutes.

7.5 Bilateral pneumothorax:

7.5.1 Once death has been confirmed, cut through the skin and muscle of the abdomen just below (caudal to) the thorax.

7.5.2 Lacerate the diaphragm with a sharp pair of scissors to ensure death.

7.6 Mechanism of action

Exsanguination causes complex interactions at the molecular, cellular, and tissue levels of dysfunctions created by a cytosolic energy failure and sustained by ischemic hypoxia. These dysfunctions interact with each other in a cause-effect relationship and a vicious circle pattern to finally result in death from cardio-circulatory arrest.

Cervical dislocation and decapitation separate the cervical vertebrae, ideally between the skull (C0), and the first cervical vertebrae (C1), completely transecting the spinal cord and disrupting blood vessels. Death is thought to be caused by cerebral ischemia and damage to the brainstem and spinal cord. Consequent exsanguination will ensure loss of consciousness and death and overcame a technical failure of the cervical dislocation.

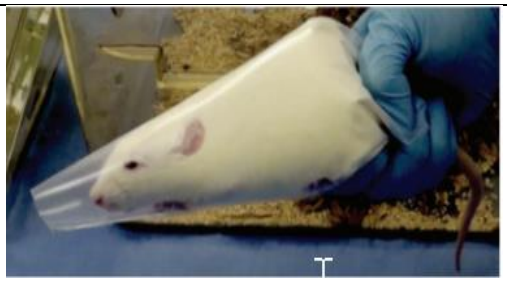
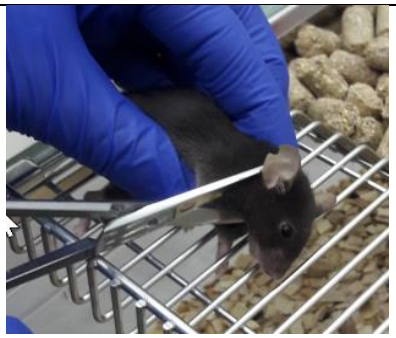
Pneumothorax will result in irreversible lung collapse.

7.7 Species

Mice and Rats

7.8 Signage

Cervical dislocation	
Guillotine	

Anesthetised rat in decapicone	
Mouse decapitation with scissors	
Exsanguination from cardiac puncture	
Bilateral pneumothorax