

**HYPOTHERMIA/CRYOANESTHESIA IN
NEONATAL RODENTS*
IACUC Standard Procedure
Effective Date: 5/11/2021**

General:

Cryoanesthesia, also referred to as deep hypothermia, is the most commonly used method to induce anesthesia in neonatal rodent species undergoing surgical procedures. This technique has been documented as safe and effective and relies on the fact that very young altricial rodents are poikilothermic and resistant to brain damage associated with cephalic circulatory arrest. Furthermore, due to their small body surface area, rapid core cooling can be achieved. Deep hypothermia provides both immobilization and mild analgesia. However, anesthetic depth during hypothermia is less controlled than during inhalant anesthesia and it can only be performed safely on neonates up to 7 days of age. Animals >P7 MUST be anesthetized with inhalant or injectable anesthetics.

Description of procedure:

1. Induction:

To induce deep hypothermia, the following guidelines must be followed:

- **Animals should NEVER be placed in direct contact with ice in order to avoid freeze damage to the skin.**
- Place pups in latex glove/sleeve or paper-lined test tube and immerse up to the neck in crushed ice and water (2°C-3°C) which requires a 5-8 minutes induction time (2-3 minutes to unconsciousness and 3-5 minutes to complete blockage of neural transmission).
- Alternatively, pups may be placed in a paper-lined tube and packed in crushed ice which requires up to 15 minutes to obtain a surgical plane of anesthesia. Crushed ice must be carefully packed to provide close contact between ice and pups.
- Anesthesia and analgesia provided by the described methods will last approximately 5 to 15 minutes but the anesthetic state may be prolonged by placing the pups on a cold pack (3°C-4°C) for another 15 minutes. It is also advised to cover the cold pack with a plastic wrap in order to keep pups dry during extended cooling. Continued absence of the pedal withdrawal reflex must be monitored frequently.
- Illumination of the surgical field should be achieved with a fiber optic surgical lamp instead of an incandescent bulb that may inadvertently cause surface warming

2. Recovery:

- After the procedure, place the recovering pups on a water circulating heating pad or under a heat lamp for at least than 5 minutes.
- Alternatively, pups can be placed in an incubator at 33°C for 20-30 minutes or in a warm humidified chamber.
- Too rapid re-warming should be avoided to minimize risks of tissue damage. Animals must be closely monitored.

- Complete recovery from cryoanesthesia may take up to 30-60 minutes.
- Supplemental oxygen might benefit recovering animals.
- Make sure that neonates are warm, pink, and capable of spontaneous movement before returning to the dam in order to minimize maternal rejection.

3. Other considerations:

- Poor administration of hypothermic anesthesia can render the pups weak and less competitive for food. In addition to the surgical manipulation experienced, weaker pups are at higher risk of maternal neglect and cannibalization and therefore require additional monitoring upon returning to the nest.
- Potential risks of hypothermia include ventricular fibrillation, tissue hypoxia and metabolic acidosis on warming.
- It is assumed that the chilling process, if done too rapidly, causes pain and distress. Nevertheless, it has been documented that pups placed in protective latex sleeves struggled and vocalized less than controls as the sleeve provides an insulating effect and might reduce cold-induced pain. Therefore, the use of a protective sleeve/tube constitutes a refinement and is required.

Literature search words required:

Literature search was performed for development of this Standard Procedure in December 2020:

<u>Key Words</u>	<u>Search Sites</u>	<u>Years Covered</u>
Cryoanesthesia, hypothermia, anesthesia, neonatal, mice, rats	PubMed	1970-2020

*In this IACUC Standard Procedure, “rodent” refers to: laboratory rats and mice.