

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_008 Anaesthesia – Cryoanaesthesia in Rodent Neonates Institutional author: Research Ethics and Integrity AEC Reviewed & Approved: 01/10/2026 SOP Expiry: 01/10/2029	Version #1.1 Page 1 of 6
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LAB_088 Anaesthesia – Cryoanaesthesia in Rodent Neonates (Expiry: October 2029)

I. OBJECTIVE

To describe the procedure for providing safe and humane anaesthesia of neonatal rodents via hypothermia (cryoanaesthesia).

NOTE

- The use of (*) indicates this statement is dependent on the facility procedures
- The use of (**) indicates this statement is dependent on AEC Approvals

II. DEFINITIONS

Analgesia - absence of pain in response to a noxious or painful stimulus.

Competent - “the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments.”

Cryoanaesthesia - The process of inducing a temporary surgical plane in neonatal rodents using severe hypothermia.

Hypothermia - A body temperature below 37°C.

Immobility - the inability to move where the muscles lay in a non-tensed loose state

P7 - Postnatal day 7

Unconsciousness - lack of awareness and perception of an animal's surroundings.

III. COMMENTS / RECOMMENDATIONS

- Cryoanaesthesia is a widely employed procedure for providing anaesthesia, immobilisation and mild analgesia for neonatal rodents up to stage P7 undergoing short (< 30 min) surgical or other painful procedures¹.
- Cryoanaesthesia exploits the heterothermy (variable, uncontrolled body temperature) exhibited by very young rodents which allows them to resist the brain damage associated with brief interruptions to circulation². Short periods of neonatal cryoanaesthesia been found to have no discernible impact on the growth of mice during development, and on their cognition into adulthood³.
- Anaesthetic depth via hypothermia is less controlled than for inhalant anaesthesia.
- Cryoanaesthesia is not appropriate for animals older than P7. Animals older than P7 **MUST** be anaesthetised with an inhalant or injectable agent (LAB_060 Rodent anaesthesia – Isoflurane; LAB_025 Rodent anaesthesia – Injectable agents)
- Neonates must be protected from direct contact with ice or ice slurries to avoid skin damage or ice burns.
- Workstations must allow neonates to be in your visual field for the duration of their anaesthetic period.
- De-sensitising the dam to the smell of gloves (e.g. latex) can help to reduce maternal rejection following the procedure. Pup should be only in contact with home nesting materials, wipes/cling film or latex gloves, throughout the procedure.

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- Potential risks of cryoanaesthesia include ventricular fibrillation, tissue hypoxia, and metabolic acidosis on rewarming.
- Poor anaesthesia or recovery may increase the risk of maternal neglect or cannibalisation.
- Cryoanaesthesia and rewarming have been associated with acute distress in neonatal rats² as evidenced by elevated ultrasonic vocalisation rates and elevated serum cortisol levels.
- Complications associated with anaesthetic and surgical procedures should be referred to the UQBR veterinarians for support (see LAB_022 UQBR Managing Adverse Health Events).

IV. SAFETY AND COMPLIANCE

- Facility and procedure appropriate PPE use is essential when handling laboratory rodents
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form. This procedure has particular risks of:
 - mouse bite injury from the dam – take appropriate care
 - musculoskeletal injury when performed regularly – consider suitable ergonomic design wherever possible

V. TRAINING CONSIDERATIONS

- All animal procedures MUST be performed by appropriately trained personnel who:
 - have been deemed to be competent in the procedure.
 - are confident in completing the procedure.
 - OR are under the direct supervision of a person who is competent.
- Training in cryoanaesthesia MUST:
 - be completed under appropriate supervision if occurring on live animals
 - include practices in LAB_002 Clean Technique for Laboratory Animal Surgery
- Workers must complete pre-requisite training:
 - LAB_006 Handling and Restraint in Mice and Neonates and/or LAB_039 Handling and Restraint in Rats and Neonates

VI. EQUIPMENT

- PPE*
- Heat mat
- Latex glove, Chux cloth + cling film, BenchKote for protecting pup from direct contact with ice/water
- Crushed ice in an esky
- Latex glove filled with an ice-water slurry and tied off.
- Timer

VII. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work
- Check cage cards, animal records and identification to ensure the correct animals are selected.

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VIII. PROCEDURE

1) Set-up

- i. Gather all equipment and prepare the work area.
- ii. Plug in a heat mat to start warming up.
- iii. Fill two eskies with ice.
- iv. Create a depression in the ice in one esky and cover with a waterproof liner (e.g. latex glove, thick Chux cloth covered with Cling film or a piece of benchkote cut to size). This 'hole' will be the induction area where the anaesthesia will take place.
The placement of a protective layer between the pups and the ice is important to avoid pain and distress from overly rapid chilling, skin damage from contact with ice and to ensure that the pups don't become wet (Figure 1).
- v. Fill a latex glove with a small amount of ice water slurry and tie it off. This will be used to cover the pup for more contact with a cold surface. Make sure it is not so heavy as to possibly restrict breathing movements.
- vi. In the other esky, place a waterproof liner over the ice to create a flat, cold surface to carry out experimental procedures under cryoanaesthesia.



Figure 1. Hypothermia anaesthesia (cryoanaesthesia) can be performed in neonatal rodents up to P7. The neonate is placed in a depression in an ice bath covered with a waterproof liner (glove, benchkote, thick Chux cloth). The neonate may then be lightly covered with a latex glove filled with an ice-water slurry to promote greater contact with the chilling surface. Figure taken from 2.

2) Procedure

- i. Remove the dam from the home cage into a separate cage, as she should not be present while handling pups to avoid maternal rejection.

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Where possible this should be done without directly touching the dam; rather, use cardboard tube to transfer dam. Provide dam with food, water and environmental enrichment such as cardboard tube from home cage to alleviate stress.

- ii. Place the pups' home cage on the warmed heat mat in preparation for recovery. Use the nesting in the home cage to create a separate recovery nest next to the pups within the home cage.
- iii. Before starting the procedure, monitor the litter of pups to check if they appear well. Ideally you will identify a milk spot in the abdomen of each which will indicate they have recently fed.
All pups should be in the nest. If there is abnormal nesting behaviour, or no milk spot, the mother may be rejecting some or all the pups, in which case consult with the UQBR vet to determine if the procedure should go ahead.
- iv. Transfer one pup into the induction area in the esky and cover it with the ice water latex glove (Figure 1). Start the timer.
Make sure the pup's skin is not in direct contact with the ice, as this can cause skin damage. The pup may need to be gently held down with the ice water glove to hold it in place while its movement slows. Do not leave weight of glove fully on the pup. It should primarily act as a cover.
- v. After approximately 1-3 minutes depending on the size of the animal, the pup should have ceased movement and breathing will have slowed considerably, to the point where it looks like the pup has stopped breathing. The pup will be unconscious at this point.
- vi. Test for a loss of pain reflex via a gentle toe pad pinch.
The pup's skin should be cold to the touch and the colour should be less pink without blood flow.
- vii. The pup can then be transferred to the flat surface on the other esky of ice for experimental procedures, such as an injection.
The anaesthesia may be maintained up to 30 minutes, but ideally pups should be anaesthetised for the shortest period necessary to complete the procedure. Use cold light sources (fibre optic, LED) to reduce risk of re-warming the pups during procedures.
- viii. If after experimental procedures, pups need an identification number, distal phalanx clipping can be done at this point while still under cryoanaesthesia. See LAB_038 Toeing in Mice and Rats.

3) Recovery

- i. To slowly warm the pup, gently hold it in a cupped gloved hand. The pup's colour should return to pink to indicate return to normal blood flow.
Warming should be done in a gradual fashion; avoid rapid warming under a heat lamp which can cause tissue damage¹. Provision of supplemental 100% oxygen may support recovery from hypoxia¹ but be aware that the flow of oxygen can also have a cooling effect.
- ii. After approximately 1 minute of warming, the pup should resume regular breathing and movement. If after 5 minutes, the pup has still not resumed breathing, it should be humanely euthanised via decapitation following LAB_009 Decapitation in Mice and Rats.
- iii. Place the pup in the recovery nest to return to normal movement. After about 10-15 minutes, the pup should be moving and should exhibit similar behaviour to prior to the procedure.
- iv. After all procedures are completed, the pups can be put back into the original nest. Remove the recovery nesting, as it may have the scent of blood on it from surgical procedures.
- v. Once all pups are fully recovered and warm to the touch, the mother can be returned to the cage.
This should be within 30-60 minutes of the initial separation from the litter depending on the length of the procedure and recovery duration. The quicker pups can be returned to the dam, the lower the chances of rejection. Observe the mother's behaviour with the pups. She should return to the nest within a few minutes.

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- vi. Monitor the pups later in the same day to ensure they are feeding and there is no maternal rejection. Continue monitoring the litter and nesting behaviour at least daily for three days following the procedure.

IX. REFERENCES AND BIBLIOGRAPHY

1. Navarro KL, Huss M, Smith JC, Sharp P, Marx JO, Pacharinsak C. Mouse Anesthesia: The Art and Science. *ILAR J.* 2021 Dec 31;62(1-2):238-273. doi: 10.1093/ilar/ilab016. PMID: 34180990; PMCID: PMC9236661.
2. Lamont KA, Boynton MH, Hickman DL, Fletcher CA, Williams MD. Acute Effects of Hypothermia and Inhalant Anesthesia on Ultrasonic Vocalizations and Neuroendocrine Markers in Neonatal Rats. *Journal of the American Association for Laboratory Animal Science.* 2024 Jan;63(1):57–66. doi:10.30802/AALAS-JAALAS-23-000008
3. Janus C, Golde T. The effect of brief neonatal cryoanesthesia on physical development and adult cognitive function in mice. *Behav Brain Res.* 2014 Feb 1;259:253-60. doi: 10.1016/j.bbr.2013.11.010. Epub 2013 Nov 14. PMID: 24239696; PMCID: PMC3883048.
4. NHMRC. (2008). Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes: The Assessment and Alleviation of Pain and Distress in Research Animals. National Health and Medical Research Council (NHMRC).
5. NHMRC. (2013). Australian code for the care and use of animals for scientific purposes, 8th edition. National Health and Medical Research Council (NHMRC).
6. Danneman PJ, Mandrell TD. Evaluation of five agents/methods for anesthesia of neonatal rats. *Lab Anim Sci.* 1997 Aug;47(4):386-95. PMID: 9306312.
7. Herrmann K, Flecknell P. Retrospective review of anesthetic and analgesic regimens used in animal research proposals. *ALTEX.* 2019;36(1):65-80. doi: 10.14573/altex.1804011. Epub 2018 Sep 14. PMID: 30222179.
8. Huss MK, Chum HH, Chang AG, Jampachairsi K, Pacharinsak C. The Physiologic Effects of Isoflurane, Sevoflurane, and Hypothermia Used for Anesthesia in Neonatal Rats (*Rattus norvegicus*). *J Am Assoc Lab Anim Sci.* 2016 Jan;55(1):83-8. PMID: 26817984; PMCID: PMC4747015.
9. University of California San Francisco. Hypothermia/Cryoanesthesia In Neonatal Rodents* IACUC Standard Procedure Effective Date: January 2024
<https://iacuc.ucsf.edu/sites/g/files/tkssra16261/files/wysiwyg/STD%20PROCEDURE%20-%20Misc%20Rodent%20Procedures%20-%20Hypothermia%20Cryoanesthesia%20in%20Neonatal%20Rodents.pdf>
10. The Kids Research Institute Australia SOP_60 CRYOANAESTHESIA IN NEONATAL MICE Approved September 2024.
11. University of Maryland, USA. Use of hypothermia as anaesthesia for neonatal rodents.
<https://research.umd.edu/sites/default/files/2021-12/Hypothermia-Anesthesia-Neonats.pdf>

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The development of this Standard Operating Procedure is duly attributed to Dr. Rebecca Cramp, whose contribution warrants formal acknowledgment. Dr Cramp is the Research Ethics and Integrity Animal Welfare Advisor (2026).

Version #	Reviewing AEC	AEC Review Date	Outcome
1.0	Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC	August 2026	Deferred Approval
1.1	Laboratory Biomedicine AEC	01/10/2026	01/10/2029

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