1. **INTRODUCTION**

Standard Operating Procedures (SOPs) provide a detailed description of commonly used procedures. SOPs offer investigators an alternative to writing detailed procedures on their protocol forms. Any deviation from the approved procedures must be clearly described and justified in the Animal Use Protocol form (AUP). Approval of the protocol indicates approval of the deviation from the SOP for that project only. A signed SOP cover page must be attached to the Animal Use Protocol form. The relevant SOP number must be referred to in the Procedures section.

2. **INFORMATION REQUIRED**

<table>
<thead>
<tr>
<th>2.1 Species/strain(s) (must refer to the Sp/strain column # of the table in “Description of animals” section in main protocol):</th>
</tr>
</thead>
<tbody>
<tr>
<td>____</td>
</tr>
<tr>
<td>2.2 □ Survival surgery □ Non-survival surgery</td>
</tr>
<tr>
<td>2.3 <strong>Anaesthesia</strong> chosen:</td>
</tr>
<tr>
<td>Procedure:</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>2.4 Details and expected duration of procedure must be in the procedure section of the main protocol <em>(section 10a of full protocol form; section 6b of renewal form)</em></td>
</tr>
<tr>
<td>2.5 Intraoperative and/or post-operative <strong>analgesia</strong> <em>(for all non-survival surgeries, intraoperative analgesia may be required, however, there is no need for post operative analgesia)</em>:</td>
</tr>
<tr>
<td>Procedure:</td>
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<td>-----------</td>
</tr>
<tr>
<td>2.6 <strong>Clinical endpoint</strong> – Clinical signs of distress requiring euthanasia: more than 20% weight loss, lack of grooming, vocalizing, ulceration, infection and surgery specific:</td>
</tr>
<tr>
<td>Immediate: hypothermia, recovery from anesthesia</td>
</tr>
<tr>
<td>In recovery: wound healing, infection, ulceration at wound site, lack of attention from mother.</td>
</tr>
<tr>
<td>Add additional endpoint criteria that would be specific to the procedures: ____</td>
</tr>
<tr>
<td>Attention must be given to wound healing, sutures, hypothermia and recovery from anaesthesia.</td>
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<tr>
<td>Frequency of monitoring: ____</td>
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<tr>
<td>2.7 Are there changes to this SOP indicated in the AUP form? □ YES □ NO</td>
</tr>
<tr>
<td>If yes, specify changes: ____</td>
</tr>
<tr>
<td>2.8 PI Signature: ___________________________ Date: ________________</td>
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</tbody>
</table>
3. **PREPARATION OF THE DAM PRIOR TO DELIVERY**
   **Purpose:** To minimize cannibalism of surgically manipulated pups
   - Handling and olfactory conditioning of dam 7-10 days prior to birthing.
   - Gentle handling for 5 minute intervals several times daily.
   - Cotton balls scented with various chemicals: hibitane (surgical skin cleanser, 70% alcohol (surgical instrument disinfectant) or gas anaesthetic (halothane, isoflurane) placed in cage for periods of 1-2 minutes daily. Never leave animal unattended at this time.

4. **ACCEPTABLE NON CHEMICAL ANAESTHETIC PROTOCOL FOR NEONATAL RODENTS**
   4.1 **Hypothermia**
   - Most commonly reported method of anaesthesia in neonatal hairless rodents. Alternative to gas anaesthesia in neonatal rodents.
   - Less control of anaesthetic depth compared to inhalant anaesthesia.
   - Very young hairless rodents function as poikilotherms and are resistant to brain damage associated with cephalic circulatory arrest. They are unable to maintain a constant internal temperature.
   - Surface cooling effective due to small surface area and body size.
   - Provides immobilization and mild analgesia (similar to local anaesthesia resulting in nerve transmission blockage).

   **Method**
   - Appropriate for short, minor surgical procedures.
   - Limited local analgesic like action.
   - Pup should be protected by latex glove or placed in paper-lined test tube to avoid freeze damage to the skin.
   - Immersion in ice water or crushed ice.
   - Induction time: 3-4 minutes.
   - Anaesthesia time: 10 minutes.
   - Recovery time: variable (up to 1 hour)
   - Pup can be kept on a cooling platform (i.e. paper covered ice pack) to provide a more constant level of hypothermia.
   - Use of a fiber optic surgical lamp rather than an incandescent one minimizes surface warming helps to maintain the hypothermia.
   - During the procedure refer to “Anaesthetic Monitoring Guidelines” outlined below. Following the procedure, refer to “Post Procedural Monitoring Guidelines outlined below.”

5. **ACCEPTABLE INJECTABLE ANAESTHETIC PROTOCOL FOR NEONATAL RATS**
   - Injectable anaesthetic agents are recommended and safest for procedures of short-term duration (less than 1 hour). If an additional dose of anaesthetic is required, use only ½ of the original dose to minimize risk of overdose and death. Duration may be unpredictable (individual variability). Recovery period may be prolonged. Hypothermia may be a concern.
   - Weigh the animal each time prior to administration of anaesthetics to ensure correct dosage.
   - Correct handling and restraining technique are crucial for accuracy of injection and to minimize stress to the animal. Prior to any injection, to check correct placement of needle and draw back on syringe. If blood, is drawn into needle hub, withdraw needle and start over again. Fresh, sterile needles and syringes must be used, and changed between animals.
   - For an IM (intramuscular) injection, the muscle located at the back of the hind leg must be isolated. Avoid penetrating too deep as bone will be encountered. In neonatal mice use a
30 gauge needle and 1 ml syringe. Maximum IM volume in a neonatal mouse = 0.020 ml. For neonatal rats, use a 27 gauge needle and a 1 ml syringe. Maximum IM volume in a neonatal rat = 0.1 ml.

- For an IP (intraperitoneal) injection, the lower abdominal cavity must be isolated. In neonatal mice use a 27 gauge needle and 1 ml syringe. Maximum IP volume in a neonatal mouse = 0.5 ml. For neonatal rats, use a 25 gauge needle and 1 ml syringe. Maximum volume in a neonatal rat = 2 ml.
- For a SC (subcutaneous) injection, locate loose skin between shoulder blades. Forming a triangle with the skin, the needle is directed into the centre of the tent and the drug injected. In neonatal mice use a 27 gauge needle and 1 ml syringe. Maximum SC volume in a neonatal mouse = 1 ml. For neonatal rats use a 25 gauge needle and 3 ml syringe. Maximum SC volume in rats = 3 ml.
- For oral dosing (per os), options include drug administration in the feed, water, or via an eyedropper. Gavage is a technique to ensure introduction of drug directly into the caudal pharynx/upper esophagus and requires technical expertise and use of proper equipment.
- During the procedure refer to “Anaesthetic Monitoring Guidelines” outlined below. Following the procedure, refer to “Post Procedural Monitoring Guidelines outlined below.”

5.1 Stock Solutions for Injectable Anaesthetics

- Xylazine (20 mg/ml)
- Hypnorm® Fentanyl 0.315 mg/ml and Fluanisone 10 mg/ml
- Diazepam 5 mg/ml.

5.2 Fentanyl-Fluanisone (Hypnorm®)

1. For dosing, the amount is reported in ml/kg body weight.
2. Fentanyl is a narcotic, see Section 7.2.
3. IP administration has been associated with a high incidence of mortality.

<table>
<thead>
<tr>
<th>TABLE 1: INJECTABLE ANAESTHETIC AGENTS FOR NEONATAL RODENTS</th>
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</thead>
<tbody>
<tr>
<td>ANAESTHETIC DRUGS</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Ketamine/Xylazine</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Ketamine/Xylazine</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Hypnorm®</td>
</tr>
</tbody>
</table>

IM= intramuscular  IP=intraperitoneal  SC=subcutaneous

6. ACCEPTABLE INHALANT ANAESTHETIC PROTOCOLS FOR NEONATAL RODENTS

- Inhalant anaesthetic gases are safe for both ultra short (<5 minutes) and prolonged (>6 hours) procedures. Inhalant anaesthetics provide a quicker induction, more stable plane of surgical anaesthesia, easier control of anaesthetic depth and smoother recovery period than injectable anaesthetics.
- Phases of anaesthesia: Induction, maintenance, recovery.
- Available inhalant gases: Isoflurane, halothane
• Ensure proper scavenging of waste anaesthetic gases, as chronic exposure can be detrimental to one’s health. Chronic exposure to halothane has been linked to hepatocellular necrosis in animals and humans.
• Isoflurane provides more rapid induction and recovery phases, and causes less cardiopulmonary depression than halothane.
• Excretion of inhalants is via the lungs.
• Materials required:
  1. Rodent non-rebreathing anaesthetic circuit equipped with a proper gas scavenging system.
  2. Adult mouse facemask adjusted for neonate
  3. Inhalant gas
  4. Oxygen tank

6.1 Induction of Anaesthesia
• Set up the anaesthetic circuit to include the chamber. The entire system should be checked daily or prior to each use. See table 2 for oxygen and inhalant gas levels.
• Place the facemask over the nose. Wait until the animal is completely relaxed before connecting facemask to the anaesthetic machine.

6.2 Maintenance of Anaesthesia
• See table 2 for oxygen and inhalant gas levels.
• See “Anaesthetic Monitoring Guidelines” outlined below.

6.3 Recovery from Anaesthesia
• See table 2 for oxygen and inhalant gas levels.
• See “Post Procedural Monitoring Guidelines” outlined below.

TABLE 2: INHALATIONAL ANAESTHETIC AGENTS FOR NEONATAL RODENTS

<table>
<thead>
<tr>
<th>PHASE OF ANAESTHESIA</th>
<th>OXYGEN (L/min)</th>
<th>HALTHANE (%)</th>
<th>ISOFLURANE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction</td>
<td>0.5-1.0</td>
<td>4-5</td>
<td>4-5</td>
</tr>
<tr>
<td>Maintenance</td>
<td>0.5-1.0</td>
<td>1-2</td>
<td>1-2</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.5 * then to 0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

• During the recovery phase, turn off inhalant gas before oxygen. If possible, allowing animal to breathe 100% oxygen for 15-20 seconds prior to breathing room air can hasten return to consciousness. In some instances, the animal’s recovery is so rapid that this will step will not be feasible.

7. ANAESTHETIC MONITORING GUIDELINES

7.1 Anaesthetic (Intra Procedural) Monitoring
• An anaesthetic record outlining drugs, doses, routes of administration and any adverse reactions should be completed and maintained for each animal for each procedure. For convenience, several animals can be entered on a single sheet.
• Monitor the animal regularly, at least every 10 minutes during the procedure and until the animal is fully ambulatory.
• Anaesthetic depth parameters:
  Respiratory rate: regular and relaxed
  Reflexes: absent withdrawal reflex
  External stimuli: no response
• Analgesic depth parameter
• Hypothermia can prolong recovery and should be prevented/minimized using a circulating hot water blanket, hot water bottles (latex gloves filled with warm water) or a heat lamp. Caution: Careful placement and close monitoring of heat sources will minimize risk of thermal injury.

7.2 Post Procedural Monitoring
• Neonatal rats and mice are extremely susceptible to hypothermia due to their high surface area: body mass ratio. For the recovery period place the animal(s) in a warm, draft free cage that is placed on a warming pad, or under a strategically located heat lamp. The animal must be able to move away from the heat source in order to prevent thermal injury. Recover pups in a cage with bedding taken from dam’s cage. Recovery can take several hours. Remove and return pups to mother as a single group.
  • Dehydration can be assessed by gently pinching a small fold of skin on the lateral thorax. If a skin tent persists then the animal is clinically dehydrated and fluid replacement must be instituted. A sterile, warm solution of physiologic saline can be administered subcutaneously at a dose of 1-2 ml per 100 grams body weight.
  • The animal must be monitored carefully until it is sternal and conscious. In order to prevent cannibalism or suffocation from its cagemates, the animal should not be returned to its home cage until it is fully ambulatory.
  • Monitor the animal’s basic biologic functions (food & water intake, urination, defecation, body weight gain) as well as clinical signs of distress (piloerection, reduced locomotion, hypothermia, decreased appetite) twice daily for at least the first week following surgery, and daily thereafter once the animal returns to complete normalcy in terms of both behaviour and appearance.
  • Do not disturb the dam and pups for several days following the procedure.

8. ACCEPTABLE ANALGESICS IN NEONATAL RODENTS
• Analgesics can be given at several different time points: prior to general anaesthesia, during general anaesthesia and once the animal has regained consciousness.
  • The most efficacious manner in which to use analgesics is prior to the “priming” of the central nervous system to the pain. Preemptive analgesia entails administering the first dose of analgesia prior to the animal returning to consciousness. The animal’s overall requirements for analgesia will be reduced as compared to an animal that receives the first analgesic dose once it is fully conscious.
  • First dose should be administered prior to animal regaining consciousness, and animal closely monitored for pain and distress for the first 24 hours post operatively. Analgesia continued for as long as is required for animal to return to preoperative appearance and behaviour.

8.1 Clinical Signs of Pain and Distress
Every effort should be made to avoid or prevent pain, distress and discomfort in the research animal. All animals undergoing a painful procedure must receive adequate analgesia during and following the procedure. Signs of inadequate analgesia, which can vary between species, may include:
  Lethargy: Inability or lack of interest in nursing
  Guarding the affected area: Vocalization
  Restlessness: Behavioural changes (aggression, withdrawal)
  Laboured breathing: Difficulty in urination
  Self mutilation: Weight loss / runting
8.2 Usage of Narcotics

8.2.1 Narcotic Drugs
- Buprenorphine
- Fentanyl

8.2.2 Permits
All researchers who wish to use controlled drugs as part of their animal experiments, must complete and submit to the Office of Controlled Substances (OCS) an “Application Form for an Exemption to use a Controlled Substance for Scientific Purposes”. A valid practice or research license is mandatory in order to purchase and use controlled drugs such as pentobarbital, ketamine and Hypnorm ®. Permits must be renewed on an annual basis. The exemption form can be downloaded from: http://www.mcgill.ca/rgo/animal/forms/
- Information can be obtained by writing or calling:
  - Office of Controlled Substances
    Drug Strategy and Controlled Substances Program
    Healthy Environments and Consumer Safety Branch
    Health Canada, A.L.: 3503B
    123 Slater St. 3rd Floor
    Ottawa, Ontario
    K1A 1B9
    (613) 952-2219 or (613) 957-1063 (phone)
    (613) 952-2196 (fax)
    email: exemption@hc-sc.gc.ca
  - Further information can be obtained by contacting the Animal Resources Centre Diagnostic and Research Support Service (DRSS) at 398-3510.

8.2.3 Storage
- All narcotics must be stored in a double locked cabinet, or preferably a wall safe, with access limited to authorized personnel only.

8.2.4 Records
- Use of all narcotics must be diligently recorded in individual, up to date narcotic logs. A narcotic log should consist of the date of usage, principal investigator possessing the research permit, drug used, species, dose (mg/kg) and volume (ml) used, balance remaining and signature of person signing out narcotic. Any residual volume must be drawn into a clean syringe, capped with a fresh needle, labeled and stored in the narcotic cabinet/safe. Controlled drugs must be disposed of according to OCS regulations. Please contact the Animal Resources Center at 398-3510 for more information.

8.3 Non Steroidal Anti Inflammatories (NSAIDS)
- Acetaminophen syrup 80 mg/ml (Tylenol Tempra ® pediatric formulation)

**TABLE 3: ANALGESIC AGENTS FOR NEONATAL RODENTS**

<table>
<thead>
<tr>
<th>ANALGESIC DRUG</th>
<th>SPECIES</th>
<th>DRUG DOSE (mg/kg)</th>
<th>ROUTE</th>
<th>DURATION (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>Mouse</td>
<td>0.05-1.0</td>
<td>SC</td>
<td>8-12</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>----------------</td>
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<td>----</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Rat</td>
<td>0.01-0.05</td>
<td>SC</td>
<td>8-12</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Rat</td>
<td>1 drop</td>
<td>PO</td>
<td>4-6</td>
</tr>
</tbody>
</table>

SC = subcutaneous  PO = oral

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Approved November 2001
Revised October 2005