7. Injectable and inhalational anaesthetic overdose euthanasia for rodents and rabbits

Support Doc. 2

Animals must not be left unattended until death is confirmed.

7.1. Material

7.1.1. Injectable anaesthetics
- Pentobarbital (Esconarkon, 300 mg/mL)
- Needles & syringes

7.1.2. Anaesthesia station
- Vaporiser (calibrated within the last 36 months), O2 source, gas scavenging system or filter, induction box

7.2. Injectable anaesthesia

7.2.1. Pentobarbital:
- 7.2.1.1. Mice and rats: inject at a dose of 150mg/kg IP (IV)
  - 7.2.1.1.1. Dilution 1.10 in rats and 1:20 in mice with NaCl or PBS
- 7.2.1.2. Rabbits: inject at a dose of 120mg/kg IV (or IP in pups until 7 PN).
  - 7.2.1.2.1. IV catheter or butterfly.

7.2.2. Animals should be placed back in their home cage or in cages in a quiet area to minimize excitement and trauma until anaesthesia followed by euthanasia is complete.

7.2.3. After deep anaesthesia is reached (loss of righting reflexes, swallow breathing) leave the animals as long as breathing has stopped.

7.3. Inhalation anaesthesia (isoflurane or sevoflurane)

7.3.1. Anaesthetic chambers should not be overloaded and need to be kept clean to minimize odours that might distress animals subsequently euthanized.

7.3.2. The anaesthetic is introduced at a maximal concentration from the vaporizer of the anaesthetic machine connected to an adequate scavenging system.

7.3.3. After deep anaesthesia is reached (loss of righting reflexes, swallow breathing) leave the animals as long as breathing has stopped.

7.4. Confirmation of death with second method

7.4.1. Check for absence of breathing and heart beat

7.4.2. Death must always be confirmed by a second technique: decapitation, exsanguination, perfusion, organ harvesting, bilateral pneumothorax, rigor mortis.

7.4.3. Dispose the cadaver

7.5. Mechanism of action

Pentobarbital overdose causes deep anaesthesia with irreversible central depression of the medullar respiratory centres followed by respiratory acidosis and secondary cardiovascular failure with heart arrest because of hypoxia and acidosis.

Inhalation anaesthetic overdose causes deep anaesthesia with central depression of the medullar respiratory centres followed by respiratory acidosis and secondary cardiovascular failure with heart arrest because of hypoxia and acidosis. Nevertheless, the washout rate of the modern low soluble anaesthetics is very fast and awakening can be possible; therefore, a second method to ensure death is mandatory.

7.6. Species

Rodents (injectable and inhalation) and rabbits (injectable).

7.7. Occupational health and safety issues

Avoid exposure to anaesthetics for workers.

Carcasses of animals euthanized with pentobarbital must be destroyed.

7.8. Pentobarbital (Esconarkon) volume calculations

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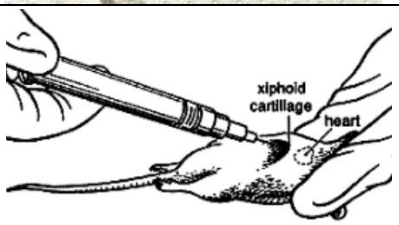
7.9. Signage

Mice and rats: inject at a dose of 150mg/kg intraperitoneally	
Animals should be placed back in their home cage or in cages in a quiet area to minimize excitement and trauma until anaesthesia followed by euthanasia is complete.	
After deep anaesthesia is reached (loss of righting reflexes, swallow breathing) leave the animals as long as breathing has stopped.	
Death must always be confirmed by a second technique: exsanguination	
Draw the maximal volume of blood (1 mL mouse, 10 mL rat)