Title: Isoflurane Anaesthesia of Mice and Rats

Background

Inhalational anaesthesia with isoflurane is quickly becoming the standard method of general anaesthesia for rats and mice used in biomedical research. It holds many advantages over injectable agents: minimal animal handling, large margin of safety, ease of anaesthetic control, low cost of anaesthetic agent, no controlled drugs, and quick recovery times. The primary disadvantages are the initial cost of equipment and the need to control human gas exposure.

Requirements

The use of isoflurane anaesthesia must be approved by the ANU Animal Ethics Committee under an animal ethics protocol; the approved protocol should stipulate the maximum anaesthesia times and frequency, percentage and flow rate of anaesthesia and details on monitoring during and post anaesthesia. Training and assessment in the use of isoflurane anaesthesia systems is required; the information provided in this document is to be used in conjunction with practical training.

To organise training please contact Training Co-ordinators at animal.services@anu.edu.au 61258197 or the Animal Ethics Committee Executive Officer at animal.ethics@anu.edu.au 61254290.

Before receiving anaesthetics training the following courses must be completed:

Animal awareness program
Introduction to working with rodents

Training details and registration can be found at https://researchservices.anu.edu.au/ori/animal/index.php
**Safety**

Users of isoflurane anaesthesia must complete a risk assessment that relates specifically to their required use.

The isoflurane Safety Data Sheet should be provided with the risk assessment and a copy should be available in the area where the anaesthesia is to be conducted. Spills kits with the ability to contain and clean up isoflurane spills should also be present in each area.

Women of childbearing age should be warned that exposure to isoflurane may have an effect on fertility and foetal development. Women who are planning pregnancy or who are pregnant may wish to request to be removed from tasks involving direct exposure such as filling isoflurane machines.

Each anaesthetic vaporiser is specific for use with one anaesthetic agent only. NEVER fill vaporisers with any other anaesthetic agent. Vaporisers are permanently and clearly labelled with the type of anaesthetic they are able to accept.

**Equipment Checklist**

Gaseous anaesthetic set ups should be inspected before each anaesthetic session.

**Medical Grade Oxygen Cylinder and Flow Meter**

Only medical grade oxygen may be used for animal anaesthesia. Turn the oxygen on at the cylinder and check the amount remaining on the regulator dial; if low ensure you have a backup cylinder ready. While the oxygen is on turn the dial on the flow meter next to the vaporiser and ensure the meter is registering an oxygen flow. For small animal work a flow meter that delivers a maximum of 1L per minute allows for more accurate flow control.

If your facility has medical grade oxygen provided through a main line this should be utilised where convenient to avoid minimise the risks involved in storing and transporting cylinders.

MSDS medical oxygen compressed
http://msds.chemalert.com/?id=21&file=0010678_001_001.pdf

Australian gas cylinder safety guidelines 2011
Isoflurane Vaporiser

Check the service date is current; vaporisers for animal use should be serviced every two years. Check the level of isoflurane and top up with the appropriate filler if required. A two way tap allows swapping delivery from the chamber to the mask more efficient.

Vaporise unit with flow meter

To fill the vaporiser you must have an isoflurane filler key; this is the only way the vaporiser can be filled.

Attach the key filler to the top of the isoflurane bottle by matching up gaps on the key with the collar on the bottle, screw it on tightly. Open the fill port and place the key in the port, once in screw the key in place. Tilt the bottle and the isoflurane will start filling the vaporiser, stop when you see liquid is touching the top line in the fill window. Release the key loosening the screw, replace the fill port plug, remove key filler from the isoflurane bottle.

They emptying port should only be used when the vaporiser requires emptying for transport.
**Induction Chamber and zero dead space mask**

![Induction Chamber and Zero dead space mask](image)

Before commencing ensure the canister is ¾ full with activated charcoal; check the date and last weight of the canister. Re-weigh and if the weight has changed by 100g the charcoal needs to be replaced. The other option is to keep a fill log with the machine, each time ½ a bottle of isoflurane is used the charcoal should be changed. To dispose of exhausted charcoal remove the lid inside a Class II cabinet or fume cupboard and place a plastic bag over the top, tipping the charcoal into the bag, seal the bag and then place another labelled bag on the outside. Your local area should have approved pails for the charcoal to be placed into for incineration. If you do not have a class II cabinet or fume cupboard you must wear a P2 mask when changing charcoal. Re-fill canister until ¾ full with fresh charcoal and return it to the machine.

**Passive charcoal canister for waste gas collection**

![Passive charcoal canister](image)

Stock containers of charcoal should be kept tightly sealed; when not in use canisters should also be sealed to prevent the charcoal becoming exhausted.

**Tubing**

Ensure tubing is connected correctly to the induction chamber/face mask and that you have a two way tap to swap between the two for delivery and to collect waste gas from both routes. If you don’t you will have to remember to disconnect and connect the correct tubing between chamber and mask use.
**Procedure**

1. Complete equipment check and set up as noted above.

2. Ensure recovery cages are set up and thermal support is provided during and after anaesthesia; for example a heat pad under the animal under anaesthesia and under the recovery cage.

3. **Induction and maintenance of anaesthesia**
   
a. ensure flow is directed to the chamber via the two way tap  
b. place the rodent in the chamber and ensure it is sealed  
c. turn the oxygen on at the bottle and flow meter, set the flow meter to deliver 1L of oxygen per minute  
d. turn on the isoflurane vaporiser to 5%  
e. monitor the rodent at all times  
f. once the rodent is recumbent turn the vaporiser to ZERO  
g. allow some pure oxygen to flow through to the chamber so that you do not exposure yourself to as much isoflurane when you open the chamber  
h. turn the two way tap to allow flow to the nose cone set up and turn the isoflurane vaporiser back on to 2%, **move the waste gas tube to nose cone**  
f. remove the rodent from the chamber and place them onto the nose cone

**Dorsal recumbency:** the nose and mouth must be inside the cone  
**Sternal recumbency:** only the nose needs to be inside the cone

g. turn the flow rate down  
Mice 0.1L/minute  
Rats 0.2L/minute

h. Adjust the vaporiser to deliver the percentage of isoflurane required to reach the depth of anaesthesia you require for your procedure

**Mice**

Minor procedures such as injections or blood sampling 2-2.5%  
Invasive procedures such as abdominal surgery 3-3.5%

**Rats**

Minor procedures such as injections or blood sampling 2-2.5%  
Invasive procedures such as abdominal surgery 3-3.5%

Corneal and toe pinch reflexes should be checked before commencing any procedure. The percentages above are a guide and may require adjustment depending on a range of factors such as whether the rodent was pre-medicated; administration of analgesia, rodent health, strain, age, weight, length of anaesthesia, thermal and fluid support.

**Conduct the procedure and always monitor the rodent: respiratory rate, body temperature, ear, tail and mucous membrane colour.**
4. Recovery

a. When the procedure has finished turn the vaporiser to zero and allow pure oxygen to flow to the animal for about 20 seconds or until you can see it is regaining consciousness

b. Turn the flow meter off and return the animal to a clean warm cage for recovery, place them on their side and ensure nothing is obstructing the nose or mouth such as bedding.

c. monitor animals until they are fully recovered

5. Moving to the next animal

a. clean the induction chamber and mask between mice if it is soiled or between experimental groups or cages to avoid cross contamination

b. do not leave cleaning residue in the chamber or on the nose cone, never use corrosive disinfectants such as bleach as this will damage the equipment

6. Completion

On completion ensure that:

a. the vaporiser is off

b. the flow meter is off and the oxygen is off at the wall or bottle, check the amount left in the bottle and replace if required or organise a backup if low

c. re-seal the charcoal canister, re-weigh and note the new weight and date on the canister

d. replace charcoal if required

e. clean the induction box and mask, remove all organic matter and soak in a non-corrosive disinfectant, allow appropriate contact time, rinse and dry

f. wipe over the tubing and vaporiser with a non corrosive disinfectant

References


www.howequipmentworks.com/physics/extras/photo/vaporiser_photo/isofluraneFiller.html

www.udmercy.edu/crna/agm/images/agm302.gif