Standard Operating Procedures	
UNIVERSITY of GUELPH **Animal Care Services**	Title: **Drop Method of Isoflurane Anesthesia**
	SOP.ACS.820. Drop Method of Isoflurane Anesthesia **Approval Date: July 12, 2024** **Revision Date:**

1. ***Purpose:*** To provide instructions for the drop method of isoflurane anesthesia when precision delivery with a vaporizer is not possible.

2. ***Responsibilities:*** Animal care staff, veterinarians, and trained individuals listed on an approved Animal Utilization Protocols (AUPs). All animal users performing procedures in animals must have successfully completed relevant training courses and mentor-facilitated training.

3. ***Introduction:***

The "drop" method of isoflurane anesthesia refers to introducing a fixed volume of liquid isoflurane into an enclosed induction chamber without a vaporizer. Vaporizers are used to precisely control gas flow and therefore anesthetic depth, typically by using oxygen as a carrier gas. However, it is not always possible to use a vaporizer, and providing oxygen and avoiding overdose is not important if the intention is to subsequently kill the animal (e.g., with CO_2 or a physical method). Using the drop method to induce anesthesia prior to death may be a refinement over using CO_2 asphyxiation or a physical method on a conscious animal.

There are now studies that validate both the effectiveness of drop method and its minimal aversiveness at 1.7% and 2.7% isoflurane in mice. However, multiple Canadian Council on Animal Care (CCAC) guidelines describe using precision vaporizers to administer isoflurane to domestic species. At this time, the drop method should only be used for terminal anesthesia when precision delivery is not possible (e.g., in the field or in spaces that will not accommodate a vaporizer) and must be justified to the ACC. This requirement may change as further research is completed. The drop method must not be used for recovery anesthetic procedures.

The following instructions are based on research performed in mice, but the same guiding principles apply to other rodents and likely other mammal species as well.

4. ***Procedures***

<u>Preparing the induction chamber:</u>

1. Designate a reusable transparent, plastic container with a secure lid that can easily be sanitized as the induction chamber. For rodents, a plastic food container with a snap lid is recommend. The container should be tall enough such that the animal cannot touch the lid.

2. Drill a small hole in the container lid to allow for the insertion of the tip of a syringe. The hole should match the syringe size you plan to use, based on the volume of the chamber (see isoflurane calculation below). For container volumes of 5 L or less, a 1 mL syringe will suffice.

3. Directly below the hole for the syringe tip, secure a piece of plastic mesh against the underside of the lid. Leave one side or corner open.

4. Slide a compressed cotton pad (from a drug store) between the mesh and the lid. This pad will absorb the isoflurane dispensed from the syringe.

5. A tea ball containing a cotton pad, hung below the hole in the lid, can alternatively be used instead of the mesh attached to the lid.

Calculating the volume of isoflurane:

1. Calculate the volume in L of the induction chamber (L x W x H in cm * 0.001 to convert cm^3 to L)

2. Calculate the temperature of the room in Kelvin (temperature in ˚C + 273.15)

3. Use the ideal gas law with P = 1 atm and R= 0.0821 to solve for the number of moles of isoflurane, then convert to mL by multiplying by the molar mass (184.5 g/mol) and the desired percent of isoflurane (1.7 – 2.7%). Refer to Appendix 1 for a sample calculation.

Anesthetizing the animal prior to a secondary killing method:

1. Ensure your anesthesia site and equipment meets human biosafety requirements for protection from volatile isoflurane.

2. Prepare a syringe with the pre-calculated volume of liquid isoflurane.

3. Assemble all necessary supplies for the secondary killing method (e.g., a pre-filled CO2 chamber).

4. Place the animal in the induction chamber and secure the lid.

5. Place the tip of the syringe with the pre-measured quantity of isoflurane in the hole in the lid and depress the plunger so that the isoflurane is deposited on the cotton pad. Do not remove the syringe.

6. Monitor the animal closely. When it becomes recumbent, gently tip the container to confirm loss of the righting reflex. If the ability to self-right and/or leg paddling is observed, wait a further 10 seconds and re-assess.

7. Once the righting reflex is lost, observe the animal for a decrease in respiratory rate (usually a further 20-30 seconds).

8. Once respiration slows, open the chamber lid and perform a toe pinch to check for the pedal withdrawal reflex. If a response is observed, close the lid and wait 30 seconds, then perform an additional toe pinch on the other paw. Alternate until there is no response.

9. Alternatively, if respiratory arrest is desired prior to a secondary method, additional isoflurane can be added to the cotton pad to facilitate anesthetic overdose once the righting reflex is lost.

10. Remove the animal from the chamber and proceed to the secondary killing method.

11. Safely allow the chamber to vent the remaining isoflurane (e.g., under a chemical fume hood).

12. Disinfect the chamber with 70% isopropyl alcohol, allow to dry, and replace the cotton pad before re-using for another animal.

5. References

Bodnar, M.J., Ratuski, A.S. & Weary, D.M. Mouse isoflurane anesthesia using the drop method. Lab Anim 57, 623-630 (2023).

Bodnar, M.J., Ratuski, A.S. & Weary, D.M. (In press). Mouse aversion to induction with isoflurane using the drop method. Lab Anim.