



THE UNIVERSITY OF TOLEDO

SUBJECT: IACUC Standard Operating Procedure for Delivering Gas Anesthetics in a Fume Hood

DATE: March 19, 2025

University of Toledo (UT) Standard Operating Procedure (SOP) for Delivering Gas Anesthetics in a Hood using Bell Jar/Open-Drop Technique

1. Introduction

This SOP is intended to provide information on appropriate procedures to be followed for delivery of inhalant anesthesia to rats, mice or other small mammals and birds. Other species must be anesthetized with inhalants via a calibrated vaporizer. The use of precision vaporizers for accurate delivery of volatile anesthetics is strongly recommended by the IACUC and must be used if a calibrated vaporizer is available or if long-duration anesthesia is needed. The Bell Jar/Open Drop technique may only be used to deliver inhalant anesthesia for very short-term procedures (i.e., 30-60 seconds) and when the following criteria are met:

- The technique is performed in a closed container that is transparent allowing the observation of the animal.
- The personnel administering the gas NEVER leaves the animal in container unattended; as few as 10 breaths can be enough for an anesthetic overdose!
- A rigid physical barrier is between the animal and the liquid anesthetic or the liquid soaked material.
- Because these conditions provide no provisions for scavenging anesthetic waste, the method must be performed inside an approved fume hood or within an area that has previously been approved by The University of Toledo, Department of Environmental Health and Radiation Safety (<https://www.utoledo.edu/depts/safety/>.)

2. Materials needed:

- Fume hood
- Bell jar or other glass container of known volume with tightly fitting lid
- Mesh platform (smooth plastic or woven wire)
- Cotton balls or gauze squares
- Conical tube(s)
- Isoflurane

3. Methods.

a. Induction-bell jar:

- Working inside a fume hood, soak a cotton ball or gauze with the appropriate amount of isoflurane (see chart below for concentration guidelines).
- Cover the isoflurane soaked cotton ball (or gauze) with a mesh platform (Figure 1). This is to prevent the rodent from coming in direct contact with the anesthetic agent.
- Place one animal at a time in the jar and close the lid tightly (Figure 3).
- Monitor the animal closely while in the jar with a focus on respiration rate.
- Once the animal has lost the righting reflex and breathing has slowed, remove the animal from the bell jar. Check the color of the mucous membranes, rate of respiration, and withdrawal reflexes. The procedure may begin if there are no reflexes but the mucus membranes and respiration appear normal.
- When the procedure is complete, euthanize the animal as outlined in the approved IACUC.

4. Anesthetic Chamber Guidelines:

a.

Concentration of Isoflurane (%)	Internal Volume of Anesthetic Chamber				
	1L	2L	3L	4L	5L
1	0.05	0.11	0.15	0.22	0.26
2	0.11	0.22	0.31	0.44	0.51
3	0.16	0.33	0.46	0.66	0.77
4	0.22	0.44	0.61	0.88	1.02
5	0.27	0.55	0.77	1.10	1.28

Volumes in the shaded area are in mL and indicate the volume of isoflurane to be applied to a cotton swab or gauze in the bell jar.

From: *"Anesthesia and Analgesia in Laboratory Animals" 3rd edition pg. 112*

b. Alternatively, isoflurane may be diluted in propylene glycol solution (1,2-Propanediol USP grade) before soaking the cotton or gauze. Dilution of isoflurane reduces the vapor in the bell jar and creates a more stable anesthetic.

- For mice, use a 20% v/v isoflurane in propylene glycol solution.
Example: To make 500 mL of 20% v/v isoflurane in propylene glycol:
{500 x 0.2=100 mL of isoflurane + 400 mL of propylene glycol}.
- For rats, use a 30% v/v isoflurane in propylene glycol solution.
Example: To make 500 mL of 30% v/v isoflurane in propylene glycol:
{500 x 0.3=150 mL isoflurane +350 mL of propylene glycol}.
- Use one mL (1cc) isoflurane/propylene glycol mixture per 500 cc volume of jar.

REFERENCES:

Dyson, M., Jirkof, P., Lofgren, J., Nunamaker, E., & Pang, D. (2023). *Anesthesia and analgesia in laboratory animals* (Third edition.). Academic Press, an imprint of Elsevier.

<https://acuc.berkeley.edu/guidelines/anesthesia.pdf>

https://iacuc.wsu.edu/documents/2016/06/ws_u_sop_3.pdf/

<https://www.purdue.edu/research/oevprp/regulatory-affairs/animal-research/docs/policies/Volatile%20Gas%20Anesthesia%20Guidelines.pdf>

Figures 1-3. Open-Drop or Bell Jar Methods.

Protect the isoflurane/propylene glycol soaked cotton ball (or gauze) from the animal with a mesh platform. Use a plastic container or Bell Jar with a tight-fitting lid.

