



Johns Hopkins University

Animal Care and Use Committee

Use of Anesthetic Gases: “Drop method”¹

PURPOSE: When performing short-term procedures with mice and rats, the “Drop Method” may be preferred for use of inhalational anesthesia as it does not require use of a vaporizer and flow meter.

OCCUPATIONAL SAFETY: To reduce occupational health risk that exists from chronic exposure to anesthetic gases, it is recommended that these procedures, as well as pouring of liquid anesthetic agents, be performed in a chemical fume hood, biosafety cabinet, downdraft table, or by use of other local exhaust methods, whenever possible. If none of this equipment is available and you wish to perform the procedure on an open bench top, the Health, Safety and Environment (HSE) office must be contacted and exposure levels measured and approved. After HSE grants approval, the ACUC office must be informed of the location so that the exception can be documented for inspection purposes. Regardless of where the procedure is performed, appropriate protective garb (gloves, lab coat, and eye protection such as face shield or goggles) should be used when dispensing liquid anesthetic agents. For information on health risks associated with gas anesthetic agents go to <http://toxnet.nlm.nih.gov/>.

METHOD: The “Drop Method” consists of permitting anesthesia liquid (e.g., halothane, isoflurane) to vaporize in close enough proximity to the animal that it becomes anesthetized. This can be accomplished by exposing the animal to the vapor in a chamber or by exposing the animal to the vapor emanating from a tube placed near its nose.

Chamber: For brief procedures, a chamber (one that permits viewing the animal) that is large enough to accommodate the animal is used to anesthetize the animal. A small amount of anesthesia liquid is placed in either a reservoir or on an absorbable material, such as gauze. The reservoir, or material, must be positioned so the animal cannot come in direct contact with the anesthesia liquid.

Two methods are described below for determining the appropriate amount of a gas anesthetic to use in the chamber.

Diluted Gas Anesthesia (PREFERRED METHOD)

Diluted gas anesthesia is the preferred method of delivery because it minimizes lethal accumulations of anesthetic in the vapor phase and provides a wider margin of safety. A mixture of 20% v/v liquid anesthetic in propylene glycol is recommended for mice, and 30% v/v liquid anesthetic in propylene glycol is recommended for rats. A general guideline for the amount of diluted mixture is ~1.0 cc for every 500 cc volume of the chamber.

Undiluted Gas Anesthesia

When using undiluted gas anesthesia, it is **extremely** important to use the appropriate amount to prevent over-anesthetizing the animal. The amount of anesthesia liquid to use should be determined based on the volume of the chamber. The table below provides a guide to the percentage of vaporization that can be achieved in relation to the ml of liquid used per liter volume of the chamber. Anesthesia usually will be adequate at 1.0 – 1.5%.

ml per liter volume of container	Equals %
0.05	1
0.10	2
0.15	3
0.20	4

Example 1: A chamber that is 10 cm wide by 10 cm long by 10 cm high = 1000 cm³ or a volume of 1.0 liter. Thus, for 1% vaporization, 0.05 ml of liquid anesthetic should be used.

Example 2: A chamber that is 8 cm wide by 10 cm long by 7 cm high = 560 cm³ or a volume of 0.560 liters (1000 cm³=1 liter). Thus, for 1% vaporization, 0.028 ml liquid anesthetic should be used.

Once the anesthetic has been loaded into the chamber, the animal is placed inside and monitored for unconsciousness. A deep plane of anesthesia is indicated by lack of a righting reflex when the chamber is tipped slightly. Once the animal is anesthetized, it can be removed from the chamber and checked to ensure an adequate depth of anesthesia before beginning the procedure. An effective method to evaluate whether you can proceed is to apply a toe pinch. If the animal responds to the toe pinch it is not adequately anesthetized and should be returned briefly to the chamber. When exposed to the air, the animal will wake up quickly so the procedure must be brief. The chamber must be sanitized between uses to prevent cross-contamination among animals.

Tube: For a slightly longer period of anesthesia, a tube (e.g., 15 ml conical tube for mice or a 50 ml conical tube for rats) can be loaded with gauze containing several drops of anesthesia liquid (slightly more if using the diluted form). The animal should be restrained and the nose held to the tube until the anesthesia takes effect. Alternatively, the animal can be anesthetized in the chamber and, after reaching the desired depth of anesthesia, the end of the tube can be placed over the nose of the animal to maintain anesthesia. It is important to assure that the gauze itself not come in contact with the animal. Care must be taken not to over-anesthetize, and thereby euthanize, the animal. By moving the tube closer or farther from the animal's nose, the level of anesthesia can be controlled. This method requires continuous monitoring of the animal.

Training in these methods can be arranged by contacting the ACUC office at acuc@jhmi.edu

¹ Approved by the JHU Animal Care and Use Committee on: October 15, 2009
minor revisions on January 28, 2010, January 31, 2018, reviewed 1/5/21