Name:	Euthanasia by Vascular Exsanguination	**Created:**	2/25/18
Number:	FSU-SOP-700.01	**Revised:**	--
Category:	Experimental Procedures	**Author(s):**	T. Schoenfeld

1.0 Purpose

This SOP describes the basic procedure for euthanasia of rodents by vascular exsanguination under anesthesia, followed by perfusion with saline and fixative, to prepare the animal for postmortem histology.

2.0 Policy

2.1 The practice of euthanasia at FSU will be performed in a manner consistent with the most recent guidelines of the American Veterinary Medical Association (AVMA; see reference 5.1).

2.2 By these guidelines, vascular exsanguination is considered appropriate only as an adjunctive method for euthanasia, following a primary method such as deep anesthesia.

2.3 Use of an inhalant anesthetic such as isofluorane is the only approved method of anesthesia in rodents at FSU (cf., SOP 600), and so is the only approved method of anesthesia to be employed in conjunction with vascular exsanguination and perfusion, preliminary to postmortem histology.

3.0 Materials

3.1 Anesthesia station (see SOP 510)

3.2 Exsanguination tray

- 3.2.1 For collection of chemical perfusate and subsequent transfer to chemical waste bottle;
- 3.2.2 With screen positioned over tray for securing anesthetized animal during procedure

3.3 Perfusion equipment

- 3.3.1 Gravity feed bottles and drip infusion set; OR Syringe pump with 60 ml syringes and infusion set
  - 3.3.1.1 Gravity feed is preferable for delivery of larger volumes to small rats
  - 3.3.1.2 Syringe pump is preferable for delivery of smaller volumes to mice
- 3.3.2 Blunted small gauge hypodermic needle to serve as a transcardiac cannula for insertion into aorta
- 3.3.3 Phosphate-buffered saline (PBS)
- 3.3.4 Fixative (e.g., 4% paraformaldehyde in PBS)

3.4 Dissection tools

- 3.4.1 Sharp scissors (small blunt tipped preferred) for ventral incision (suitable for cutting through skin, muscle, and rib cage)
- 3.4.2 Sharp scissors (small pointed tipped preferred) for opening heart
- 3.4.3 Mouse-tooth forceps

3.4.4 Hemostats
3.5 Personal protective equipment
3.5.1 Double gloves
3.5.2 Lab coat and sleeve covers

4.0 Procedure

4.1 Arrange anesthesia station, exsanguination tray, perfusion equipment, and dissection tools within the exhausted biosafety cabinet (BSC; class II type B2) in the Procedure Room (SCI 101D). Fixatives should be prepared in a chemical fume hood in one of the wet labs, and then dispensed to a gravity feed bottle or syringe only within the BSC. Be sure to turn on the exhaust ventilator.
4.2 Set up the perfusion equipment so that each solution is ready for delivery, with air bubbles expelled along the entire line.
4.3 Bring the animal into the Procedure Room from the holding room in its home cage, or transfer a group-housed animal directly into the gas anesthesia induction chamber.
4.4 Anesthetize the animal in the induction chamber, following SOP 510, verifying that it has reached a surgical plane of anesthesia by loss of eye blink and toe pinch (pedal) reflexes.
4.5 Secure the animal on its back, by its limbs, to the screen over the exsanguination tray, and continue anesthesia by delivering gas through a nose cone.
4.6 Then open the ventral body cavity, using sharp scissors to make a longitudinal incision along the ventral midline, starting with the abdominal skin and muscle wall, then continuing through the rib cage along the sternum, along with the overlying skin and thoracic muscle, taking care to keep the scissors away from the heart and great vessels. Snip the diaphragm along the caudal rib cage, and then reflect the rib cage to either side, secured with hemostats.
4.7 With heart exposed, open the PBS infusion line slightly so that a very slow flow of fluid is expelled from the cannula. Then, snip open the right atrium and the left ventricle, and carefully insert the cannula through the left ventricle until the tip is seen entering the aorta. Hold the cannula in place with a hemostat clamped on the left ventricle, then open the PBS line further to deliver fluid at a flow rate of 3-5 ml/min, roughly half the nominal cardiac output in a mouse or even less than that in a small rat (see reference 5.2). The liver should turn pale after a few minutes if the PBS perfusion is successfully replacing vascular blood. The delivery of gas anesthetic can be turned off at this point.
4.8 After 5 min, switch to the fixative line and continue the perfusion at the same rate for 15-30 min. Clamp the abdominal aorta if the tissue of interest for postmortem histology is confined to the head region.
4.9 Stop flow of fixative and proceed to prepare tissues for postmortem procedures.
4.9.1 Store the residual carcass in a labeled bag at -20 deg C, to be picked up by a contracted disposal service.

4.10 Clean up
- 4.10.1 Flush both PBS and fixative lines with RO water.
- 4.10.2 Transfer collected perfusate to a waste container and arrange for disposal by technical staff.
- 4.10.3 Rinse all tools and affected equipment with RO water and air dry.
- 4.10.4 Return all cleaned and dried tools and equipment to storage, leaving work areas (BSC, counter, surgical table) clear for the next user.

5.0 References

5.1 AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

5.2 Physiological parameters. http://rmi-pharmacokinetics.com/Physiological_parameters

5.3 Transcardiac Perfusion (McGill University) https://www.mcgill.ca/research/files/research/305-_transcardiac_perfusion_-_jan_2018_0.pdf

SOP REVISION HISTORY

VERSION #	APPROVED	DETAILS
700.01	m/d/yy	Authored by T. Schoenfeld