

**Research Animal Standard Operating Procedures (SOP) must meet the following criteria:**

1. Describe procedures or activities involving research animal(s) common to more than one research project.
2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.
3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and
4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

<b>Name of Procedure</b>	CO <sup>2</sup> Asphyxiation- Euthanasia dam, foetal and neonatal	
<b>Species</b>	Guinea Pig	
<b>ACEC</b>	<b>Reference</b>	SOP#148- Mar23 - CO2 asphyxiation- euthanasia dam, foetal and neonatal- guinea pig
	<b>Author</b>	Jon Hirst
	<b>Version</b>	1.2
	<b>Date approved</b>	24 March 2023
	<b>Date for review</b>	24 March 2026
	<b>Procedure classification</b>	2
<b>Ethical considerations</b>	<ol style="list-style-type: none"> <li>1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes.</li> <li>2. The procedure must be performed according to current best practice to support the wellbeing of the animal.</li> <li>3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent.</li> </ol>	

## Details

### 1. Purpose

To sacrifice maternal, foetal, or juvenile guinea pigs by CO<sub>2</sub> asphyxiation.

### 2. Description of procedure

#### **EQUIPMENT & MATERIALS:**

- Compressed medical grade CO<sub>2</sub> gas cylinder with rubber hose connected to regulator
- Regulator and flow meter for gas cylinder
- Euthanasia chamber – plastic container with a hole in top to avoid pressure and also a hole in the side of the container (towards the base) for connection of rubber hose from gas cylinder
- Scissors
- Scalpel Blade
- Nitrile gloves
- Guinea pig/s to be sacrificed
- Benchcoat
- Black body bags

Prior to euthanasia ensure work area is set up for post-mortem: Place benchcoat on bench and prepare bench with necessary equipment including scissors, blades, tubes for blood and tissue collection, liquid nitrogen, syringes and needles.

#### **METHOD: Pregnant Dam**

- a) Remove the pregnant dam from its home cage using two hands by placing the palm of one of your hands around the animal's ribs without squeezing and at the same time supporting the posterior and rear legs with your other hand. Hold the pregnant dam close to your abdomen while still supporting the posterior and rear legs.
- b) Place the guinea pig to be euthanased in a clean euthanasia chamber, in a separate room away from other guinea pigs and insert rubber hose into small hole at the bottom side.
- c) Turn on the CO<sub>2</sub> mains ensuring the flow rate (on the flow meter) is set to 3-7L/min (30-70% of chamber volume per minute).
  - i) *As advised by the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition - An optimal flow rate for CO<sub>2</sub> euthanasia systems should displace 30% to 70% of the chamber or cage volume/min, with the understanding that there is 'potential for increased distress due to dyspnea at lower flow rates or mucous membrane pain associated with flow rates at the high ends of this range. However, as there is no clear evidence of a flow rate that optimally minimizes both pain and distress for all species, sexes, and genetic backgrounds, veterinarians should use their professional judgment to determine which flow rate is appropriate for their circumstances'.*
  - ii) In our experience, we suggest a flow rate of 6L/min to be optimal.

- d) Observe the animal for signs of loss of (behavioural) consciousness (loss of righting reflex, animal recumbent; approximately 3 minutes). Once unconscious, flow rate can be adjusted to rate of +10L/min (maximal rate of the flow meter is 12.5L/min).
- e) Leave the animals in the chamber for a further 7 minutes to ensure death.
- f) Turn off CO<sub>2</sub> and remove animal.
- g) Verify death by lack of corneal and pedal reflexes and cardiac pulse.
- h) Use secondary measures to assure death by incising the abdomen immediately below the ribs and puncturing the diaphragm to induce bilateral pneumothorax. Place the dam on its abdomen and sever the cervical section of the spinal cord just below the base of the skull using a scalpel blade.
- i) Proceed with fluid and tissue collection
- j) Dispose of body in black body bag provided by the RSU and freeze.
- k) Before reusing the euthanasia chamber ensure it has been thoroughly cleaned and dried.

#### **METHOD: Foetal Guinea pigs**

- a) Remove the pregnant dam from its home cage using two hands by placing the palm of one of your hands around the animal's ribs without squeezing and at the same time supporting the posterior and rear legs with your other hand. Hold the pregnant dam close to your abdomen while still supporting the posterior and rear legs.
- b) Place the guinea pig to be euthanased in a clean euthanasia chamber, in a separate room away from other guinea pigs and insert rubber hose into small hole at the bottom side.
- c) Turn on the CO<sub>2</sub> mains ensuring the flow rate (on the flow meter) is set to 3-7L/min (30-70% of chamber volume per minute).
  - i) In our experience, we suggest a flow rate of 6L/min to be optimal (see above).
- d) Observe the animal for signs of loss of (behavioural) consciousness (loss of righting reflex, animal recumbent; approximately 3 minutes). Once unconscious, flow rate can be adjusted to rate of +10L/min (the maximal rate of the flow meter is 12.5L/min).
- e) Leave the animals in the chamber for a further 7 minutes to ensure death.
- f) Turn off CO<sub>2</sub> and remove animal.
- g) Verify death by lack of corneal and pedal reflexes and cardiac pulse.
- h) Use secondary measures to assure death by incising the abdomen immediately below the ribs and puncturing the diaphragm to induce bilateral pneumothorax. Place the dam on its abdomen and sever the cervical section of the spinal cord just below the base of the skull using a scalpel blade.
- i) Use forceps to pull up skin of mid abdomen of the pregnant dam, cut using blunt ended large scissors, blunt dissect and continue cutting down the midline, pick up muscle layer and cut in a similar fashion making sure not to perforate the gut.
- j) Gently remove uterus from abdomen. Incise uterus between pups to avoid accidentally inflicting wound to foetus and gently push pups out of uterus.
- k) Upon removal from the uterus verify death of by lack of reflexes and cardiac pulse.
- l) Use secondary measures to assure death by incising the abdomen immediately below the ribs and puncturing the diaphragm to induce a bilateral pneumothorax and severing the cervical section of the spinal cord just below the base of the skull using a scalpel blade.
- m) Proceed with tissue collection.
- n) Dispose of bodies in black body bag provided by the RSU and freeze.
- o) Before reusing the euthanasia chamber ensure it has been thoroughly cleaned.

**METHOD: Neonatal/Juvenile Guinea pigs**

- a) Individually remove juvenile guinea pigs from the home cage using two hands by placing the palm of one of your hands around the juvenile guinea pigs ribs without squeezing and at the same time supporting the posterior and rear legs with your other hand. Hold the juvenile guinea pig close to your abdomen while still supporting the posterior and rear legs.
- b) Place the guinea pig/s to be sacrificed in a clean euthanasia chamber, in a separate room away from other guinea pigs and insert rubber hose into small hole at the bottom side. Do not overcrowd.
- c) Turn on the CO<sub>2</sub> mains ensuring the flow rate (on the flow meter) is set to 3-7L/min (30-70% of chamber volume per minute).
  - i) In our experience, we suggest a flow rate of 6L/min to be optimal (see above)
- d) Observe the animal for signs of loss of (behavioural) consciousness (loss of righting reflex, animal recumbent; approximately 3 minutes). Once unconscious, flow rate can be adjusted to rate of +10L/min (the maximal rate of the flow meter is 12.5L/min).
- e) Leave the animals in the chamber for 10 minutes to ensure death.
- f) Turn of CO<sub>2</sub> and remove animals.
- g) Verify death by lack of corneal and pedal reflexes and cardiac pulse.
- h) Use secondary measures to assure death by incising the abdomen immediately below the ribs and puncturing the diaphragm to induce bilateral pneumothorax and severing the cervical section of the spinal cord just below the base of the skull using a scalpel blade.
- i) Proceed with tissue collection.
- j) Dispose of body in black body bag provided by the RSU and freeze.
- k) Before reusing the euthanasia chamber ensure it has been thoroughly cleaned and dried.

**Quick ref guide for CO2 inhalation euthanasia flow rates:**

Size	4L	10L	20L	44L
<i>Initial flow rate</i>	2.4L/min	6L/min	12L/min	26.4L/min
	Identify animals are unconscious before increasing flow (approx. 3mins)			
<i>Subsequent flow rate</i>	+4L/min	+10L/min	Max flow	Max flow

**References**

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.  
[https://www.avma.org/sites/default/files/2020-01/2020\\_Euthanasia\\_Final\\_1-15-20.pdf](https://www.avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf)

ACEC Chair

