

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.

Name of Procedure	Euthanasia by intravenous sodium pentobarbitone	
Species	All species	
ACEC	Reference	SOP#76 - Euthanasia by intravenous sodium pentobarbitone
	Author	Jenny Smart
	Version	1.4
	Date approved	25 August 2023
	Date for review	31 August 2026
	Procedure classification 1. Observation involving minor interference 2. Animal unconscious without recovery 3. Minor conscious intervention 4. Minor surgery with recovery 5. Major surgery with recovery 6. Minor physiological challenge 7. Major physiological challenge	2
Ethical considerations	1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. 2. The procedure must be performed according to current best practice to support the wellbeing of the animal. 3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent.	

Details

Purpose

To describe the euthanasia of research animals by injection of an overdose of sodium pentobarbitone intravenously.

Barbiturates depress the central nervous system, with an overdose resulting in rapid loss of consciousness, deep anaesthesia, apnoea and then cardiac arrest. They induce euthanasia smoothly and with minimal discomfort to the animal. The euthanasia dose is typically three times the anaesthetic dose. Sodium pentobarbitone is the most commonly used barbiturate for laboratory rodents.

Description of procedure

EQUIPMENT

1. Sodium pentobarbitone as euthanasia solution (300-325 mg/ml)
2. Syringe of a size suitable to hold the volume of sodium pentobarbitone needed.
3. Hypodermic needle of an appropriate size e.g. 29G for mice and 25G for rats
4. Clippers
5. Device for calculating weight of animals e.g. scales
6. Antiseptic such as 70% ethanol in water or chlorhexidine in 70% ethanol in water
7. Cotton gauze swabs

NOTE

1. The dose of sodium pentobarbitone for euthanasia is at least 200mg/kg for rodents.
2. This procedure must only be performed by people who are experienced in performing intravenous injections in the species being euthanased.

PROCEDURE

1. Euthanasia must be carried out in a quiet place away from other animals.
2. Ensure that only one animal is dealt with at a time. Any other animals must be kept in a separate room.
3. Attach the hypodermic needle to the syringe, swab the top of the sodium pentobarbitone bottle with antiseptic, insert the needle through the rubber diaphragm and draw up the correct volume of sodium pentobarbitone.
4. Do not recap needle, set aside syringe and needle.
5. Catch and restrain the animal as approved by the Animal Care and Ethics Committee in the approved protocol.
6. See table below for appropriate veins in different species. Applying antiseptic with a cotton gauze swab may make the vein more obvious in some species.
7. Insert the needle into the vein and inject the full volume of sodium pentobarbitone as quickly as possible. Slow injections may result in the animal entering an 'excitement' phase characterised by struggling and vocalisation.
8. Confirm death via lack of heartbeat before disposal.

Species	Approximate Weight (Adult)	Suitable veins	Minimum Volume of 300mg/ml Sodium pentobarbitone euthanasia solution*
Mouse	30g	Tail	20ul
Rat	450g	Tail	0.3ml

References

2020 AVMA Guidelines for the Euthanasia of animals.
<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>

ACEC Chair

