

**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>	Euthanasia by intraperitoneal sodium pentobarbitone	
<b>Species</b>	Rats, mice, guinea pigs	
<b>ACEC</b>	<b>Reference</b>	SOP#75 – Jul 23 - Euthanasia by intraperitoneal sodium pentobarbitone - rodents Appendix 1
	<b>Author</b>	Jenny Smart
	<b>Version</b>	1.4
	<b>Date approved</b>	24 July 2020
	<b>Date for review</b>	24 July 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

### Purpose

To describe the euthanasia of rat, mice and guinea pigs by injection of an overdose of sodium pentobarbitone injected intraperitoneally.

Barbituates depress the central nervous system, with an overdose resulting in rapid loss of consciousness, deep anaesthesia, apnea and then cardiac arrest. They induce euthanasia smoothly and with minimal discomfort to the animal. The preferred route of administration is intravenous, however, where intravenous injection is difficult such as in the small rodents, intraperitoneal injection is an acceptable alternative.

### 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. 29-25G for mice; 25-23G for rats, guinea pigs.
4. Device for calculating weight of animals e.g. scales or weigh tape.
5. Antiseptic such as 70% ethanol in water or chlorhexidine in 70% ethanol in water
6. Cotton gauze swabs
7. Water for injection.

#### **NOTE**

1. The dose of sodium pentobarbitone for euthanasia is at least 200mg/kg for rodents and 150 mg/kg for larger species.
2. This procedure must only be performed by people who are experienced in performing intraperitoneal 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 or pen.
3. Euthanasia solutions generally have a high pH and can be irritant if injected intraperitoneally. If using a euthanasia solution dilute the concentration to 60mg/ml using water for injection. 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. Insert the needle intraperitoneally, draw back on the plunger to confirm that the needle is not in the bladder or intestine and then 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.
7. Confirm death via lack of heart beat before disposal.

8. Intraperitoneal injections may accidentally be administered into an organ within the abdominal cavity. If this occurs, death may be prolonged. In such cases a physical method of ensuring death must be used when the animal is deeply unconscious. Cervical dislocation in mice or opening the thorax or decapitation in rats and guinea pigs is a suitable way of ensuring death before disposal.

Species	Approximate Weight (Adult)	Minimum Volume of 300mg/ml Sodium pentobarbitone euthanasia solution*
Mouse	30g	20ul
Rat	450g	0.3ml
Guinea Pig	1 kg	0.7 ml

\* Multiple by 5 when diluted to 60mg/ml

## References

2020 AVMA Guidelines for the Euthanasia of animals.  
[https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx?utm\\_source=prettyurl&utm\\_medium=web&utm\\_campaign=redirect&utm\\_term=issues-animal\\_welfare-euthanasia-pdf](https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx?utm_source=prettyurl&utm_medium=web&utm_campaign=redirect&utm_term=issues-animal_welfare-euthanasia-pdf)

## ACEC Chair

