

<b>Title</b>	Anaesthesia of rats for surgical procedures		
<b>SOP no</b>	SOP_Viv_Anim 2	<b>Issue no</b>	3
<b>Issue date</b>	23-06-2014	<b>Revision date</b>	24-06-2016
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1. COMPILATION AND AUTHORISATION

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Action	Designated Person	Signature	Date
Compiled by:	Mrs A Fick	<i>A Fick</i>	20/06/2014
Authorised by:	Mr CJJ Bester	<i>CJJ Bester</i>	20-06-2014

2. DISTRIBUTION

**Controlled Document**

Department	Name	Signature	Date
PCDDP QA Manager	L Scholtz	<i>L Scholtz</i>	24-06-2014
Head: Vivarium	CJJ Bester	<i>CJJ Bester</i>	24.06.2014

3. DOCUMENT HISTORY

Date	Issue no	Reason for revision
30-08-2012	Draft1	First draft of SOP based on initial process
5-06-2013	1	First issue of SOP
17-02-2014	2	Addition of test principal
23-06-2014	3	Training Removed

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**4. POLICY and OBJECTIVE**

This SOP establishes the techniques required to anaesthetise rats for surgical procedures

**5. SCOPE**

This SOP establishes the techniques required to anaesthetise rats for surgical procedures in the theatre of the GLP animal unit.

**6. REFERENCE DOCUMENTS**

- SANS 10386:2008 The Care and Use of Animals for Scientific Purposes
- SOP\_Viv\_Eqp\_Opr 2 – Weighing of Animals and Materials
- SOP\_All\_Stor 1 – Storage and Use of Scheduled Pharmaceuticals and Controlled Substances
- SOP\_Viv\_Anim 3 – Intraperitoneal Injection in Laboratory Animals

**7. ABBREVIATIONS and/or DEFINITIONS**

**7.1. ABBREVIATIONS**

Abbreviation	Description
GLP	Good laboratory practice
N/A	Not applicable
SOP	Standard operating procedure
ACP	Acepromazine
HCL	Hydrochloric acid
MSD	Material safety data sheet

**7.2. DEFINITIONS**

Term	Definition
Anaesthetic	Drug or compound used to achieve a plane of insensitisation
i.p. (intra-peritoneal)	Abdominal injection by penetration of integument, muscle layers and peritoneum from the exterior.
SANS	South African National Standard

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## 8. RESPONSIBILITIES

This SOP must be followed by all staff in the Vivarium who have been assessed by the head as competent to apply the procedure below, following a period of training and experience under a trained operator. This SOP applies only to staff employed in the Vivarium and is not intended as an authorisation for any other personnel or students. Staff in the process of gaining competence may undertake the procedure if guided by a trained member of the vivarium.

## 9. TEST PRINCIPAL

N/A

## 10. MATERIALS and EQUIPMENT

- Anaesthetic apparatus
- Isoflurane
- Nose cone
- Cotton wool
- 26G needle
- Ketamine HCl (100mg/ml)
- Xylazine (Rompun; 100mg/ml)
- Acepromazine maleate (ACP; 2mg/ml)
- Sterile water
- Stoppered injectable bottle (amber if ACP is used)
- Heating pad or heat lamp

## 11. SAFETY

- 11.1. All personnel performing anaesthesia must be appropriately trained and the training recorded
- 11.2. Isoflurane must only be used in a properly ventilated room, preferably in a fume cupboard.
- 11.3. Isoflurane anaesthesia must not be performed by or in the presence of a pregnant woman.
- 11.4. All chemicals, etc. must be handled appropriately and not in contradiction of any MSD sheet notification.

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## 12. PROCEDURES

### 12.1. GENERAL


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- a) The animal's identity is verified by examining its tattoo, dyed marking, ear notching or other identification.
- b) Gather all necessary equipment, and wash hands and put on gloves prior to the onset of the procedure.
- c) The animal is weighed according to (SOP\_Viv\_Eqp\_Opr 2) if necessary to calculate a dose
- d) Depending on the duration of anaesthesia required and specific requirements of the study (some anaesthetic drugs will be contra-indicated in certain protocols), three different anaesthetic regimens are available:
  - Isoflurane for terminal procedures such as cardiac puncture (11.2.)
  - Ketamine/Xylazine for medium-term anaesthesia (up to 30 min) (11.3.)
  - ACP/Ketamine/Xylazine for long-term anaesthesia (up to 5 hrs.) (11.4.)
- e) The animal needs to be closely monitored and kept warm using a heat pad or heat lamp while under anaesthesia and during recovery from anaesthesia. Extreme care must be taken with the use of heat lamps as they have the potential to burn the skin of the animal.

### 12.2. Isoflurane anaesthesia with nose cone

- a) 0.5 to 1 ml of isoflurane is poured onto a cotton wool pad and placed inside the nose cone. The animal is restrained by hand and the nose cone applied. The rat will hold its breath for a while, but once it has taken the first breath of the anaesthetic, it will fall asleep. The nose cone remains on the animal throughout the procedure.
- b) A record should be maintained of volumes withdrawn from the bottle. (SOP\_All\_Stor 1) This record may be in the form of an adhesive label attached to it.

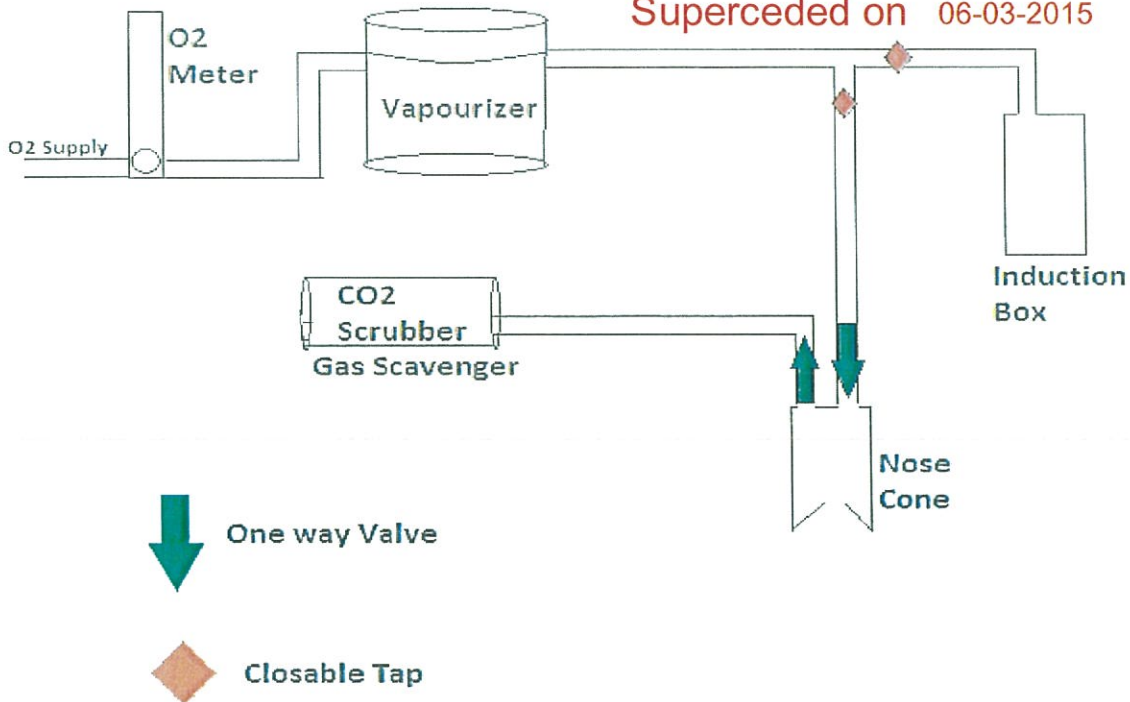
			
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12.3. Isoflurane anaesthesia with anaesthetic apparatus (See diagram)

- a) The apparatus consists of an O<sub>2</sub> supply, O<sub>2</sub> meter, vapourizer, induction box, nose cone and CO<sub>2</sub> scrubber.
- b) The reservoir of the vapourizer is filled with isoflurane. The vapourizer is first set on 4% to give the set concentration in the induction box which is used to induce anaesthesia.
- c) Oxygen from the supply flows through the oxygen meter, through the vapourizer into the induction box.
- d) Closable taps are used to regulate the flow of the anaesthetic into the induction box.
- e) The rat is put in the box and its breathing and reflexes closely monitored.
- f) Once no reflexes are observed and its breathing has slowed, the rat can be taken out of the induction box and placed on the nose cone.
- g) The concentration of the anaesthetic gas is changed to 2%.
- h) Once on the nose cone air is circulated through a CO<sub>2</sub> scrubber to remove the CO<sub>2</sub>.
- i) Once the procedure is done, the rat can be removed from the nose cone.
- j) The rat must be closely monitored, kept warm and given an analgesic if necessary until fully recovered.

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#### 12.4. Ketamine/Xylazine anaesthesia

- The two drugs are mixed at a rate of 10mg ketamine to one mg of xylazine. A clean stoppered bottle that is labelled appropriately can be used to hold the mixture. The two drugs are injected into the bottle, each in a volume that adheres to the ratio indicated above. The mixture is stable and has a shelf life of one month, provided that none of the two drugs expires before the month has expired
- A record should be maintained of volumes withdrawn from each of the two bottles, the total volume prepared in the compound bottle, and the use-by-date of the mixture. This record may be in the form of an adhesive label attached to the appropriate bottles.
- The prepared solution will be approximately 91mg/ml ketamine HCl and 9 mg/ml xylazine. This is injected i.p. (according to SOP\_Viv\_Anim 3.) at a dose of 1ml/kg live weight.

#### 12.5. ACP/Ketamine/Xylazine anaesthesia

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- a) The three drugs are admixed at a rate of two parts of ACP to five parts of ketamine to one part of xylazine to two parts of sterile water (by volume). A clean amber stoppered bottle or pill bottle that is labelled appropriately can be used to hold the compound. The three drugs and the water are injected into the bottle, each in a volume that adheres to the ratio indicated above. The admixture is stable and has a shelf life of one month, provided that none of the three drugs expires before the month expires.

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- b) A record should be maintained of volumes withdrawn from each of the four bottles, the total volume prepared in the compound bottle, and the use-by-date of the admixture. This record may be in the form of an adhesive label attached to the appropriate bottles.
- c) The prepared solution will be 0.4mg/ml acepromazine maleate, 50mg/ml ketamine HCl and 10 mg/ml xylazine. This is injected i.p. (according to SOP\_Viv\_Anim 3) at a dose of 1ml/kg live weight.

#### 12.6. Recovery

- a) If the procedure is being used for recovery surgery, a post-operable analgesic should be used.
- b) Rats that were anaesthetized must be monitored until fully recovered and water and food intake are normal.

#### 13. ADDENDUM

N/A