

THE UNIVERSITY OF WESTERN ONTARIO  
ANIMAL CARE AND VETERINARY SERVICES

Standard Operating Procedure # 320-02

EUTHANASIA

1 INTRODUCTION

Standard Operating Procedures (SOPs) provide a detailed description of procedures to be followed unless alternate procedures have been outlined in an Application to Use Animals. If an investigator wishes to proceed differently from an approved SOP, the changes must be itemized in the application. To assist the Animal Use Subcommittee in protocol review, the reasons for deviating from the standard procedure should also be included.

Approval of the protocol indicates approval of the deviation from the SOP for that project only.

Euthanasia means "painless death". Numerous methods are/have been used to kill animals. However, only certain methods may be used in a given species. The following will briefly outline acceptable procedures and the species in which they may be used. If an individual has any question that the animal is dead the thoracic cavity must be opened and the animal observed for a minimum of five minutes.

2 PROCEDURE

2.1 CARBON DIOXIDE

2.1.1 Species: Mice, rats, hamsters, small guinea pigs, gerbils - not suitable for neonates.

2.1.2 Comments:

2.1.2.1 Performed properly animals will first be ANAESTHETIZED with low levels of CO<sub>2</sub> and then, ONCE UNCONSCIOUS will be asphyxiated by high concentrations.

2.1.2.2 CO<sub>2</sub> gas from a cylinder is cold, equipment should deliver the gas through a large bore tubing which will serve to warm the gas.

2.1.2.3 Nose rubbing may be caused by the drying action of the CO<sub>2</sub>.

2.1.2.4 Do not use dry ice. The gas concentration can not be controlled and it is cold.

2.1.3 Procedure:

2.1.3.1 Place the animals in the CO<sub>2</sub> chamber then turn the gas on VERY SLOWLY.

2.1.3.2 The animals should not become agitated. Respiration rate will increase due to increase in blood CO<sub>2</sub> levels.

2.1.3.3 Once the animal has lost consciousness, increase flow rates until respirations cease.

2.1.3.4 At this point, blood may be collected via cardiac puncture.

2.1.3.5 If blood is not being collected leave the animal in the chamber for approximately one minute following cessation of respiration. Removing the animal immediately may stimulate respiration.

2.2 PENTOBARBITAL

2.2.1 Species: May be used in all species

2.2.2 Comments:

2.2.2.1 Pentobarbital is controlled by the Bureau of Dangerous Drugs, a license is required

to hold this drug. Stringent documentation is required. Alternatively, the drug may be purchased, on an individual dosage basis, from ACVS.

2.2.3 Procedure:

2.2.3.1 Inject by an acceptable intravenous route or intraperitoneally. Dosage: 110mg/kg. MUST NOT BE GIVEN INTRAMUSCULARLY OR INTRAARTERIALY (EXCEPT THE CAROTID ARTERIES).

2.3 CERVICAL DISLOCATION NOT ACCEPTABLE FOR AMPHIBIANS OR REPTILES

2.3.1 Species: UNLESS THE ANIMALS IS PREANAESTHETIZED THIS PROCEDURE IS ONLY ALLOWED WITH SCIENTIFIC JUSTIFICATION AND PRIOR APPROVAL OF THE ANIMAL CARE COMMITTEE. THE PROCEDURE MUST BE PERFORMED BY AN EXPERIENCED PERSON, APPROVED BY THE ANIMAL CARE COMMITTEE. For use in mice, small rats and birds.

2.3.2 Procedure: Mammals

2.3.2.1 Experience must be gained on dead and/or anaesthetized animals.

2.3.2.2 Grasp the animal by the base of the tail and place it on a firm surface (counter top).

2.3.2.3 Press a rod across the neck at the base of the skull (Atlanto-occipital joint) or alternatively use fingers. This has the advantage of allowing the operator to feel the separation of the joint.

2.3.2.4 With the other hand grasp either the base of the tail or the hind limbs.

2.3.2.5 Quickly pull back and slightly up on the animal while resisting this motion with the hand holding the rod, or with the fingers.

2.3.2.6 When learning/practising this procedure ensure that cervical dislocation has occurred by dissection or palpation.

2.3.3 Procedure: Birds

2.3.3.1 Special instructions are required. Contact ACVS.

2.4 DECAPITATION/GUILLOTINE

2.4.1 Species: UNLESS ANIMAL IS PREANAESTHETIZED THE PROCEDURE IS ONLY ALLOWED WITH SCIENTIFIC JUSTIFICATION AND WITH PRIOR APPROVAL OF THE ANIMAL CARE COMMITTEE. THE PROCEDURE MUST BE PERFORMED BY AN EXPERIENCED PERSON, APPROVED BY THE ANIMAL CARE COMMITTEE. For use in small rodents, young rabbits, birds.

2.4.2 Procedure:

2.4.2.1 Except for neonatal rodents and small birds, a properly constructed guillotine must be used.

2.4.2.2 Equipment must be kept in exceptional working order.

2.4.2.3 A sharp pair of scissors may be used for neonatal (<1wk) rodents (except guinea pigs).

- 2.4.2.4 The individual performing the procedure must be experienced in the gentle handling and restraint of the species.
- 2.4.2.5 The individual performing the procedure must gain experience using dead or anaesthetized animals.
- 2.4.2.6 The procedure must not be performed in the presence of other animals.
- 2.4.2.7 The animal is removed from the cage and immediately decapitated.
- 2.4.2.8 The head is severed at the highest possible level (C1-C2).
- 2.4.2.9 NOT ACCEPTABLE FOR EUTHANASIA OF AMPHIBIANS OR REPTILES UNLESS PREVIOUSLY ANAESTHETISED.

2.5 EXSANGUINATION NOTE: THIS PROCEDURE MAY ONLY BE PERFORMED ON FULLY ANAESTHETIZED ANIMALS.

2.5.1 Species: Any

2.5.2 Procedure:

2.5.2.1 The procedure will depend on the species involved. In smaller animals it is usually accomplished by percutaneous cardiac puncture. Contact ACVS for details.

2.6 AMPHIBIANS AND REPTILES

2.6.1 For methods other than pentobarbital or methods where the animal is previously anaesthetized contact ACVS.

### 3 SUMMARY

3.1 It is imperative that animals are euthanized using only acceptable methods. It is also essential that the animals are handled gently while they are being euthanized.

### 4 REFERENCES

- 4.1 Canadian Council on Animal Care. Guide to the Care and Use of Experimental Animals. Volumes 1 and 2, 1984.
- 4.2 Canadian Council on Animal Care. Euthanasia Methods. Communication, January 1988.
- 4.3 Canadian Council on Animal Care. Cervical Dislocation. Resource, 1:2 1988.
- 4.4 Euthanasia of Amphibians and Reptiles. Report of a Joint UFAW/WSPA Working Party, 1989.
- 4.5 Government of Ontario. Animals for Research Act. Regulation 18, Section 28, 1-2.
- 4.6 Smith AW (Chair): Report on the AVMA Panel on Euthanasia. JAVMA, 3:252, 1986.
- 4.7 Rowsell, HC: Euthanasia: Acceptable and Unacceptable Methods of Killing. In Rollin BE (ed): The Experimental Animal in Biomedical Research. Boca Raton, Fla. CRC Press 1988.
- 4.8 Universities Federation for Animal Welfare. Practical Instructions-Fish. Draft Copy for 4th Edition. Killing of Animals.

### 5 REFEREES

- 5.1 Bailey, M: Director, Animal Care & Veterinary Services, University of Western Ontario, London, Ontario.
- 5.2 Latt, R: Director, Animal Resources Centre, McGill University, Montreal, Quebec
- 5.3 Rowsell, H: Executive Director, Canadian Council of Animal Care, Ottawa, Ontario

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