



### Standard Operating Procedure

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Title: <b>Euthanasia of Rabbits</b>		
Prepared by: (typed) (b)(6)	QA Review: (typed) (b)(6)	Approving Authority: (typed) (b)(6)
(Signature/Date) (b)(6) 10 Dec 1998	(Inits./Date) (b)(6) 10 Dec 98	(Signature/Date) (b)(6) 10 Dec 98

#### I. PURPOSE

The purpose of this SOP is to describe procedures for the humane euthanasia of rabbits.

#### II. RESPONSIBILITY

It is the responsibility of any person euthanizing a rabbit to follow the procedures as described. Study Directors are responsible for designing animal use protocols so that the method of euthanasia chosen is considered acceptable by the American Veterinary Medical Association Panel on Euthanasia. If no acceptable method can be used because of scientific reasons, the Study Director must provide justification for use of an alternate method to the Laboratory Animal Care and Use Committee.

#### III. MATERIALS

- Needle or butterfly collection set and syringe
- Euthanasia solution and/or anesthetic
- Needle and syringe for blood collection if necessary
- Hatbox and liner or other appropriate container for carcass disposal
- Autoclave indicator tape
- Sharps container

#### IV. EQUIPMENT

Rabbit restrainer if needed

#### V. PROCEDURE

##### A. General

1. Two methods for the euthanasia of rabbits are commonly used at USAMRIID: barbiturate overdose and exsanguination subsequent to anesthesia. If neither of these methods is scientifically acceptable, consult the Veterinary Medicine Division for an alternative.

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2. Euthanasia should not be performed in the presence of (i.e. in the same room with) other animals.

3. No technician or caretaker may euthanize an animal unless directed to do so by the Study Director, Investigator, or attending Veterinarian.

4. Prior to euthanizing an animal, determine whether a necropsy is required, by checking the protocol, asking the Study Director, Investigator, or attending Veterinarian. If a necropsy will be performed, notify the duty Pathologist that an animal requiring a necropsy is about to be euthanized.

5. Verify the rabbit's identification before performing the euthanasia.

#### B. Barbiturate overdose

1. A barbiturate overdose with a sodium pentobarbital euthanasia solution can be administered intravenously (IV) through the marginal ear vein or via intracardiac (IC) injection of an already anesthetized rabbit. Intraperitoneal injection is also acceptable but IV or IC administration is preferable since it is more rapid.

2. To insure rapid death, draw 125-150 mg/kg (0.3-0.4 ml/kg) of euthanasia solution into a syringe. Use a 23 gauge or larger needle whenever possible because the solution is thick.

3. Inject the euthanasia solution in the marginal ear vein (refer to SOP AC-AC-09-20, Giving intravenous injections to small animals and nonhuman primates) or the peritoneal cavity (refer to SOP AC-09-30, Giving intraperitoneal injections to small animals and non-human primates). To inject the euthanasia solution IC:

a. Anesthetize the rabbit as described in SOP AC-11-12 (Injectable Anesthesia for Rabbits), then place it in dorsal recumbency.

b. Palpate the heart with the thumb and second finger; then position the index finger over the xiphoid process of the sternum, as a reference point.

c. Insert the needle just under and to the left of the animal's xiphoid process at a 30-45 degree angle from the horizontal plane of the abdomen, keeping the needle and syringe parallel to the midline.

d. Quickly thrust the needle forward through the skin and diaphragm, and into the chest, maintaining slight negative pressure on the plunger.

e. Once the heart is penetrated, blood will readily flow into the hub of the syringe. Pulling back too hard on the plunger may collapse the heart.

f. If no blood appears, the initial thrust of the needle into the chest may have pushed the heart to the side, the angle of approach may have been too steep, or the needle may have passed through the heart into the lung tissues or thoracic space. Slowly withdraw the needle from the chest until blood flows, or repeat the entire procedure.

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g. Once the needle is in the heart, inject the euthanasia solution as a single bolus and remove the needle and syringe.

4. Confirm that the rabbit is dead by the absence of a heart beat 5 minutes or more after euthanasia. If the rabbit still has a heart beat, inject additional euthanasia solution IC as described above.

5. Discard all used sharps in a sharps container.

#### C. Exsanguination subsequent to anesthesia

**NOTE:** Exsanguination of an unanesthetized rabbit is not acceptable.

1. Anesthetize the rabbit as required by the protocol and/or described in SOP AC-11-12 (Injectable Anesthesia for Rabbits).

2. Once the animal is anesthetized, withdraw blood via cardiac puncture as described in SOP AC-13-20 (Collection of blood from rabbits). Greater than 30% of the total blood volume must be withdrawn to result in the death of the rabbit. Therefore, **at least** 20 ml of blood per kg of body weight should be removed.

3. Confirm that the rabbit is dead by the absence of a heart beat 5 minutes or more after euthanasia. If the rabbit still has a heart beat, remove more blood or inject a barbiturate overdose as described above.

4. Discard all used sharps in sharps containers.

## VI. REFERENCES

SOP AC-09-20 Giving intravenous injections to small animals and non-human primates  
SOP AC-09-30 Giving intraperitoneal injections to small animals and non-human primates  
SOP AC-11-12 Injectable Anesthesia for Rabbits  
SOP AC-13-20 Collection of blood from rabbits  
Report of the AVMA Panel on Euthanasia, Journal of the AVMA, 1993, Vol. 202(2): 229-249.

## VII. DISTRIBUTION

See SOP AD-01-02 Distribution of Standard Operating Procedures

## VIII. REVISION HISTORY

None