



Euthanasia – Standard Operating Procedures

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1. Overview

Purpose

The Drexel University Institutional Animal Care and Use Committee (IACUC) has established this procedure to assure compliant and humane methods of euthanasia consistent with The Guide for the Care and Use of Laboratory Animals and AVMA Guidelines on Euthanasia (<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>).

2. Definitions

Euthanasia is defined as “ending the life of an individual animal in a way that minimizes or eliminates pain and distress”.

3. General Euthanasia

- Animals must be continuously observed during the procedure.
- Once the euthanasia process has begun, the animals must not be left alone until after confirmation of death.
- Any method of euthanasia where the animal is likely to emit distress vocalizations or pheromones that other animals can hear or smell should be performed in a room or area separated from all other animals
- Euthanasia should be conducted in the home cage to minimize animal distress and anxiety. If home cage euthanasia cannot be practiced, the process must minimize pain and distress and the chambers must be cleaned between each use to eliminate the detection of distress odors.
- Euthanasia chambers must be transparent so animals may be observed.
- In accordance with The Guide for the Care and Use of Laboratory Animals, a method of confirmation of euthanasia must be performed on all animals by a secondary method of euthanasia.
- The carcass should be disposed of in a bag and placed in a designated cooler or freezer.

4. Guidelines

The following lists are meant to be used as guidelines for common methods and procedures by species. All euthanasia methods (primary and secondary/confirmation) must be approved through IACUC before use.



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4.1 Mice/Rats/Small Rodents

Acceptable Primary Methods

Agent	Route of Administration	Notes
Carbon Dioxide	Inhalation	<ul style="list-style-type: none"> Only compressed CO₂ from a gas cylinder controlled by a regulator and flow meter is acceptable. The lid must fit properly and be vented at the top to allow the air to be displaced. The Chamber must not be pre-charged. The flow rate must be 50% of the chamber volume/minute. CO₂ Flow must be maintained for 60 seconds after visual confirmation of respiratory cessation. In instances where residual CO₂ is expected, procedures should be in place to ensure removal of residual CO₂ gas between euthanasia sessions. Maximum number of animals per cage is 5 mice or 3 rats.
Anesthetic Overdose Chamber followed by physical means of euthanasia (Isoflurane, sevoflurane)	Inhalation	<ul style="list-style-type: none"> Must use a precision vaporizer with a sealed induction chamber and waste gas scavenger. The vaporizer should start at 3-4% and be administered slowly up to 4.5% (isoflurane) or 6.5% (Sevoflurane). 'Deep anesthesia must be verified by lack of response to noxious stimuli'. Under deep anesthesia, the animal must be euthanized by cervical dislocation, decapitation, perfusion or exsanguination.
Anesthetic/Sedative Overdose, Injection (IP) (Euthasol, pentobarbital, ketamine/xylazine combinations)	Injection (IV or IP)	<ul style="list-style-type: none"> Animals must be monitored until lack of heartbeat is noted for at least 60 seconds. Must ensure that the dose provided is an overdose (x3 anesthesia dose). Most agents are DEA regulated. (http://drexel.edu/facilities/healthSafety/labSafety/DEA%20Controlled%20Substances/)
Perfusion under anesthesia	Physical	<ul style="list-style-type: none"> Depth of anesthesia must be verified and continue until the heart stops. Should be performed under a chemical hood if required by EHS.
Exsanguination under anesthesia	Physical	<ul style="list-style-type: none"> Depth of anesthesia must be verified and continue until respiration has ceased for at least 3 minutes. Rapid removal of blood can be obtained via cardiac venipuncture or severing major vessels
Decapitation (Conscious rodents) Only performed by specially trained personnel, prior IACUC approval required	Physical	<ul style="list-style-type: none"> Must have prior IACUC approval. Special training required. Special rodent guillotines must be used for adults. Equipment must be kept clean, in good condition with sharp blades. A sharpening log must be kept.



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<p>Cervical Dislocation- (conscious rodents) Only performed by specially trained personnel on rats/mice less than 200 grams, prior IACUC approval required</p>	<p>Physical</p>	<ul style="list-style-type: none"> • Must have prior IACUC approval. • Special training required.
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Acceptable Secondary/Confirmation Methods

- Bilateral Thoracotomy
- Decapitation
- Cervical Dislocation
- Dissection of a major organ
- Exsanguination

Acceptable Primary Methods for Fetus and Neonate Mice/Rats

Age	Method	Notes
Fetus less than 14 days old	Dam euthanasia or removal of fetus	<ul style="list-style-type: none"> • Method of euthanasia for dam should ensure rapid cerebral anoxia to the fetus, with minimal disturbance to the uterine milieu, minimizing fetal arousal. • Unnecessary to remove the fetuses after dam is euthanized. • Removal of fetus results in rapid death due to blood loss.
Fetus greater than or equal to 15 days old	Dam euthanasia or removal of fetus	<ul style="list-style-type: none"> • Method of euthanasia for dam should ensure rapid cerebral anoxia to the fetus with minimal disturbance to the uterine milieu minimizing fetal arousal. • Unnecessary to remove fetus after dam is euthanized. • If fetal tissue is to be collected, removal of the uterus or intact amniotic sac must occur. The cessation of blood flow will result in rapid death. Once death has occurred, the fetus can be removed. • If fetal breathing occurs, a physical method of euthanasia from the approved neonate methods must be performed, e.g., decapitation with scissors.
Fetus 15 days to birth	Decapitation with scissors	<ul style="list-style-type: none"> • Scissors must be sharp.
Fetus and neonates up to 5 days of age	Rapid freezing in liquid nitrogen (liquid nitrogen immersion)	<ul style="list-style-type: none"> • Mouse and rat fetuses and neonates < 5 days of age may be quickly killed by rapid freezing in liquid nitrogen. • If at any point the fetus is allowed to breathe it must be decapitated.
Up to 7 days old	Hypothermia followed by decapitation	<ul style="list-style-type: none"> • Animals must not come in direct contact with ice or precooled surfaces. • Maintain on ice until movement ceases, then perform decapitation with sharp scissors.
Up to 10 days old	Injectable Anesthetic Overdose	<ul style="list-style-type: none"> • See information for mice/rat adults
Up to 10 days old	Carbon Dioxide	<ul style="list-style-type: none"> • Not recommended • May take up to 50 minutes for death to occur



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		<ul style="list-style-type: none"> • Second method required once unconscious • Same process as adults
Up to 10 days old	Inhalant Anesthetic Overdose	<ul style="list-style-type: none"> • Place neonates in induction chamber • Time to achieve anesthesia will take longer than for adult rodents • Second method required once unconscious • Same process as adults
Up to 10 days old	Decapitation (conscious)	<ul style="list-style-type: none"> • Sharp scissors or disposable scalpel may be used • Equipment must be kept clean, in good condition with sharp blades. • A sharpening log must be kept. • Must be justified and approved by the IACUC.

4.2 Rabbits

Most inhalation methods are unacceptable without premedication due to the tendency to struggle and not breathe when presented with unfamiliar or unpleasant stimuli.

Acceptable Primary Methods for Rabbits

Agent	Route of Administration	Notes
Anesthetic Overdose (Barbiturates)	Injection Venous access via the ear IP if necessary however care must be taken not to inject into a hollow organ	<ul style="list-style-type: none"> • Sedation may be necessary for animals not used to handling • Restraining cage can be used to calm the animal and immobilize • Animals must be monitored until lack of heartbeat is noted for at least 60 seconds • Must ensure that the dose provided is an overdose (x3 anesthesia dose) or use commercial euthanasia solution recommended dose • Most agents are DEA regulated (http://drexel.edu/facilities/healthSafety/labSafety/DEA%20Controlled%20Substances/)
Inhalant Anesthetic Overdose followed by physical means of euthanasia	Inhalation	<ul style="list-style-type: none"> • Not a recommended method as a sole method of euthanasia • Must be pre-medicated with a sedative • Must be properly restrained to prevent kicking • Must use a precision vaporizer with a sealed induction chamber and waste gas scavenger • Once deep anesthesia is maintained, euthanasia can be performed by perfusion or exsanguination
Perfusion under anesthesia	Physical	<ul style="list-style-type: none"> • Depth of anesthesia must be verified and continued until the heart stops • Should be performed under a chemical hood if required by EHS



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Exsanguination under anesthesia	Physical	<ul style="list-style-type: none"> Depth of anesthesia must be verified and continue until respiration has ceased for at least 3 minutes Rapid removal of blood can be obtained via cardiac venipuncture or severing major vessels
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Acceptable Secondary/Confirmation Methods

- Bilateral Thoracotomy
- Decapitation
- Cervical Dislocation
- Tissue Perfusion
- Dissection of a major organ
- Exsanguination

4.3 Swine

Acceptable Primary Methods

Agent	Route of Administration	Notes:
Anesthetic Overdose (Euthasol, barbiturates)	IV injection Ip if necessary	<ul style="list-style-type: none"> • Sedation may be necessary for animals not used to handling • Animals must be monitored until lack of heartbeat is noted for at least 60 seconds • Must ensure that the dose provided is an overdose (x3 anesthesia dose) or commercial euthanasia solution recommended dose • These agents are DEA regulated

Acceptable Secondary/Confirmation Methods

- Bilateral Thoracotomy
- Decapitation
- Cervical Dislocation
- Tissue Perfusion
- Dissection of a major organ
- Diaphragm Interruption
- Exsanguination

4.4 Amphibians

Acceptable Primary Methods

Agent	Route of Administration	Notes:
Tricaine Methanesulfonate (MS-222)	Tank immersion	<ul style="list-style-type: none"> • Commercially available FDA approved brand should be used • MS222 solutions for tank immersion and injections are acidic and irritating and must be buffered with sodium bicarbonate to a physiologically



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Not for use in “captured and released” animals of Field Studies.	<p>Injected into lymph sacs</p> <p>Injected intracolelcomic</p>	<ul style="list-style-type: none"> • appropriate pH before use. • Animals must be monitored until lack of heartbeat is noted for at least 60 seconds • Must ensure that the dose provided is an overdose • MS-222 stock solutions should be utilized the same day as preparation per vendor recommendation. • Special handling and preparation of MS222 required because it is a chemical hazard and requires biosafety approval prior to use.
Physical method under anesthesia	Physical	<ul style="list-style-type: none"> • Decapitation, Pithing, rapid chilling, and thermal shock are all acceptable methods once a deep anesthesia is ensured.

Acceptable Secondary/Confirmation Methods

- Decapitation
- Double Pithing
- Rapid chilling
- Tissue Perfusion
- Dissecting of a major organ

4.5 Fish

Acceptable Primary Methods

Agent	Route of Administration	Notes:
<p>Triciane Methanesulfonate (MS-222)</p> <p>Not for use in “captured and release” animals of Field Studies.</p>	Tank immersion	<ul style="list-style-type: none"> • Commercially available FDA approved brand should be used • MS222 solutions for tank immersion are acidic and irritating and must be buffered with sodium bicarbonate to a normal pH before use. • The immersion water should have adequate levels of dissolved oxygen • Animals must be monitored until lack of heartbeat is noted for at least 60 seconds • Must ensure that the dose provided is an overdose • Soluble in both fresh and salt water and can be used for a wide variety of species • MS-222 stock solutions are utilized the same day as preparation per vendor recommendation. • Special handling and preparation of MS222 required because it is a chemical hazard and requires biosafety approval prior to use.
Clove Oil, Isoeugenol, and eugenol	Total immersion	<ul style="list-style-type: none"> • Must ensure that the dose provided is an overdose • Should be left in solution for a minimum of 10 minutes after cessation of opercular movement • Recommended that products with standardized, known concentrations of essential oils be used so that accurate dosing can occur



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Rapid Chilling Small-bodied tropical and subtropical stenothermic species only	Physical	<ul style="list-style-type: none"> • Adult zebrafish should be exposed for a minimum of 10 minutes • Fry 3-7 days after fertilization should be exposed for a minimum of 20 minutes • Unreliable for embryos
Diluted sodium or calcium hypochlorite solutions for embryos- fry 3 days after fertilization	Tank immersion	<ul style="list-style-type: none"> • Should follow one of the other methods to ensure embryotic lethality

Acceptable Secondary/Confirmation Methods

- Decapitation
- Freezing
- Cervical Dislocation

5. Responsibilities

5.1 Drexel University IACUC Responsibilities

The Drexel University IACUC and the IACUC Office are responsible for maintaining this guidance document, training, and monitoring. All exceptions to this procedure must be approved by the IACUC. For inquiries regarding these procedures, please contact the Director of Animal Welfare, a part of the Office for Research & Innovation (ORI), or the Attending Veterinarian.

5.2 Principal Investigator Responsibilities

Euthanasia is defined as “ending the life of an individual animal in a way that minimizes or eliminates pain and distress”. To be compliant with this definition, all personnel performing euthanasia must be properly trained on the chosen technique of euthanasia. Staff must also be familiar with normal behavior and pain assessment of the species in question. It is the Investigator’s responsibility to ensure all staff performing euthanasia are properly trained, familiar with the target species, and follow policy accordingly. Training is available through Drexel ULAR or the Animal Welfare Education Specialist.

The human–research animal bond positively impacts quality of life for a variety of research animals, but those caring for the animals often experience euthanasia- related stress symptoms. Researchers should be aware that conducting euthanasia can be distressful to students and staff and be prepared to discuss these issues.



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6. Resources

- [AALAS COST OF CARING: RECOGNIZING HUMAN EMOTIONS IN THE CARE OF LABORATORY ANIMALS](#)
- [AAALAC FAQ - Carbon Dioxide for Rodent Euthanasia](#)
- [NIH Animal Research Advisory Committee \(ARAC\) Guidelines](#)

7. Revisions

Edition 001/Effective Date: 05/10/2017 – Original Document

Edition 001/Update Date: 06/2021 - Original Document

Edition 002/Review/Revision Date: 06/12/2024 and Effective Date: 06/26/2024 – Revised Document.

- Updated formatting to new template.
- Changed title to “Euthanasia Procedures”
- Addition of Definition Section
- Section 3 – removal of “double kill” reference and will only be referred to as secondary/confirmation method of euthanasia.
- Section 4.1: Anesthetic Overdose, Chamber (Isoflurane, sevoflurane) Agent Column– Addition of “followed by physical means of euthanasia”
- Section 4.1: Anesthetic Overdose, Chamber (Isoflurane, sevoflurane) Notes Column – Removal of “May need to be exposed for prolonged time periods to ensure death”, “A secondary method must be used to confirm death after the animal becomes unconsciousness” “Nitrous oxide should not be used alone, but may be used in conjunction with other inhaled anesthetic” “The anesthetic flow must be maintained for 60 seconds after visual confirmation of respiratory cessation”
- Section 4.1: Anesthetic Overdose, Chamber (Isoflurane, sevoflurane) Notes Column – Addition of “Depth of anesthesia must be verified by lack of response to noxious stimuli'. Under deep anesthesia, the animal must be euthanized by cervical dislocation, decapitation, perfusion or exsanguination”
- Section 4.1 – Removal of “Anesthetic Overdose, Desiccator Jar/Open Drop” as a recommended euthanasia method. *The use of this euthanasia method must be justified and approved by the IACUC before use.*
- Section 4.1: Decapitation Agent Column – Change of “live” to “conscious rodents”
- Section 4.1: Cervical Dislocation Agent Column – Addition of “conscious rodents”
- Section 4.1: Fetus and Neonate Age Column – Change 2nd Fetus 15 days to birth to “Fetus and neonates up to 5 days of age”. Method Column - Removal of “while anesthetized”. Notes Column - Addition “Mouse and rat fetuses and neonates < 5 days of age may be quickly killed by rapid freezing in liquid nitrogen.” Removal “Anesthesia may be effectively induced by hypothermia of the fetus, which can be achieved by submerging the fetus (with the amniotic sac



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intact) in cold (4- 8°C/35-39°F) physiological saline until the fetus becomes completely immobile”

- Section 4.1: Fetus and Neonate Up to 7 days old– Addition of “followed by decapitation” to Method Column
- Section 4.1: Fetus and Neonate Up to 10 days old Carbon Dioxide – Addition of May take up to 50 minutes “for death to occur” Second method required “once unconscious” to Notes Column
- Section 4.1: Fetus and Neonate Up to 10 days old Inhalant Anesthetic Overdose – Removal of “Not recommended” “May take up to 50 minutes”. Addition of “Place neonates in induction chamber” “Time to achieve anesthesia will take longer than for adult rodents” Second method required “once unconscious” to Notes Column.
- Section 4.1: Fetus and Neonate Up to 10 days old Decapitation. Addition of “or disposable scalpel” to Notes Column.
- Section 4.2: Anesthetic Overdose (Barbiturates) – Addition of “or use commercial euthanasia solution recommended dose” to Notes Column.
- Section 4.2 – Removal of “Carbon Dioxide under sedation” as a recommended euthanasia method.
- Section 4.2: Inhalant Anesthetic Overdose – Agent Column - Addition of “followed by physical means of euthanasia” Notes Column – Addition of Not a recommended method “as a sole method of euthanasia” “Once deep anesthesia is maintained, euthanasia can be performed by perfusion or exsanguination” Removal of “May need to be exposed for prolonged time periods to ensure death” “The vaporizer should start at 3-4% and administered slowly up to 4.5% (isoflurane) or 6.5% (Sevoflurane)” “The anesthetic flow must be maintained for 60 seconds after visual confirmation of respiratory cessation” “If euthanasia is occurring while under anesthesia, animal must be tested for responsiveness before proceeding.” “Nitrous oxide should not be used alone, but may be used in conjunction with other inhaled anesthetic” “Secondary method must be used”
- Section 4.3: Anesthetic Overdose (Euthasol, barbiturates) - Addition of “or use commercial euthanasia solution recommended dose” to Notes Column
- Section 4.4 & 4.5: Tricaine Methanesulfonate (MS-222) Notes Column – Removal of “which includes buffer”. Addition of “MS222 solutions for tank immersion and injections are acidic and irritating and must be buffered with sodium bicarbonate to a physiologically appropriate pH before use.” “MS-222 stock solutions should be utilized the same day as preparation per vendor recommendation.” “Special handling and preparation of MS222 required because it is a chemical hazard and requires biosafety approval prior to use.”
- Section 4.5: Tricaine Methanesulfonate (MS-222) Notes Column – Addition of “The immersion water should have adequate levels of dissolved oxygen”
- Section 4.5 Secondary/Confirmation Method – Change or Rapid Chilling to Freezing.
- Section 5.1 – Addition of Drexel IACUC responsibilities



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- Section 5.2 – Addition of compassion fatigue awareness and engagement
- Section 6 – Addition of AALAS COST OF CARING: RECOGNIZING HUMAN EMOTIONS IN THE CARE OF LABORATORY ANIMALS