

DREXEL UNIVERSITY ANIMAL CARE AND USE COMMITTEE POLICY ON EUTHANSIA

OBJECTIVE: Drexel University Animal Care and Use Committee has established this policy 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>).

RESPONSIBILITY: 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.

General Euthanasia notes:

- 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 if transportation stress can be minimized.
- When possible, 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 should be cleaned between each use.
- Euthanasia chambers must be transparent so animals may be observed.
- The euthanasia chamber must be cleaned between animals so as to eliminate the detection of distress odors.
- 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 (double kill).
- The carcass should be disposed of in a bag and placed in a designated freezer.

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

Mice/Rats/Small Rodents

Acceptable Primary Methods for Mice/Rats/Small Rodents

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 (Isoflurane, sevoflurane)	Inhalation	<ul style="list-style-type: none"> • Must use a precision vaporizer with a sealed induction chamber and waste gas scavenger • 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. • 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

Anesthetic Overdose, Desiccator Jar/Open Drop Only rats/mice less than 200 grams	Inhalation	<ul style="list-style-type: none"> • The animal may need to be exposed for prolonged time periods to ensure death • The animal must remain in the jar for at least 60 seconds after a visual confirmation of respiratory cessation • A desiccator jar may not be used for any procedure other than euthanasia • The jar must not be overcrowded. If using conical tube, only one animal may be euthanized at a time.
Anesthetic/Sedative Overdose, Injection (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 (Live) 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 sharpen log must be kept.
Cervical Dislocation- Only performed by specially trained personnel on rats/mice less than 200 grams, prior IACUC approval required	Physical	<ul style="list-style-type: none"> • Must have prior IACUC approval • Special training required

Acceptable Confirmation Methods for Mice/Rats

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

Fetus and Neonate Mice/Rats (https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_euthanasia_pup.pdf)

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 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 the 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, ie decapitation with scissors
Fetus 15 days to birth	Decapitation with scissors	<ul style="list-style-type: none"> • Scissors must be sharp
Fetus 15 days to birth	Rapid freezing in liquid nitrogen while anesthetized (liquid nitrogen immersion)	<ul style="list-style-type: none"> • Anesthesia may be effectively induced by hypothermia of the fetus, which can be achieved by submerging the fetus (with the amniotic sac intact) in cold (4-8°C/35-39°F) physiological saline until the fetus becomes completely immobile • If at any point the fetus is allowed to breath it must be decapitated
Up to 7 days old	Hypothermia	<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 • Second method required • Same process as adults
Up to 10 days old	Inhalant Anesthetic Overdose	<ul style="list-style-type: none"> • Not recommended • May take up to 50 minutes • Second method required • Same process as adults
Up to 10 days old	Decapitation (conscious)	<ul style="list-style-type: none"> • Sharp scissors may be used • Equipment must be kept clean, in good condition with sharp blades. • A sharpen log must be kept. • Must be justified and approved by the IACUC

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	<u>Route of Administration</u>	<u>Notes</u>
Anesthetic Overdose (Barbituates)	Injection Venous access via the ear IP if necessary however care must be taken	<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 60seconds • 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/)

	not to inject into a hollow organ	
Carbon Dioxide under sedation	Inhalation	<ul style="list-style-type: none"> • Not a recommended method • Must be pre-medicated with a sedative • Must be properly restrained to prevent kicking • 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 should be 50% of the chamber volume/minute • CO2 Flow must be maintained for 60 seconds after visual confirmation of respiratory cessation • Secondary method must be used
Inhalant Anesthetic Overdose	Inhalation	<ul style="list-style-type: none"> • Not a recommended method • 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 • 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
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
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Acceptable Confirmation Methods for Rabbits

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

Cats/Dogs/Swine

Acceptable primary methods for Cats/Dogs/Swine

Agent	Route of Administration	Notes:
Anesthetic Overdose (Euthasol, barbituates)	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) • Most agents are DEA regulated

Acceptable Confirmation Methods for Cats/Dogs/Swine

- Bilateral Thoracotomy
- Decapitation

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

Amphibians

Acceptable primary methods for Amphibians

Agent	Route of Administration	Notes:
Tricaine Methanesulfonate (MS-222) Not for use in “captured and released” animals of Field Studies.	Tank immersion Injected into lymph sacs Injected intracolelcomic	<ul style="list-style-type: none"> • Commercially available FDA approved brand should be used which includes buffer • Animals must be monitored until lack of heartbeat is noted for at least 60 seconds • Must ensure that the dose provided is an overdose • Special handling and preparation required
Physical method under anesthesia	Physical	<ul style="list-style-type: none"> • Decapitation, Pithing, rapid chilling, and thermal shock are all acceptable methods one a deep anesthesia is ensured.

Acceptable Confirmation Methods for Amphibians:

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

Fish

Agent	Route of Administration	Notes:
Tricaine Methanesulfonate (MS-222) Not for use in “captured and released” animals of Field Studies.	Tank immersion	<ul style="list-style-type: none"> Commercially available FDA approved brand should be used which includes buffer Animals must be monitored until lack of heartbeat is noted for at least 60 seconds Must ensure that the dose provided is an overdose Special handling and preparation required Soluble in both fresh and salt water and can be used for a wide variety of species
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
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 solution for embryos-fry 3 days after fertilization	Tank immersion	<ul style="list-style-type: none"> Should follow one of the other methods to ensure embryonic lethality

Acceptable Confirmation Methods for Fish

- Decapitation
- Rapid Chilling
- Cervical Dislocation

Reference:

<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>

<https://oacu.oir.nih.gov/animal-research-advisory-committee-guidelines>

<http://www.medschool.umaryland.edu/iacuc/Guidelines/Recommended-Methods-of-Euthanasia/>

<https://research.wayne.edu/iacuc/euthanasiaofrodentfetusesandneonates>

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