



Internal Regulation Title	Acceptable Euthanasia Methods				
Date Last Revised	12/14/2021	Date Created	08/03/2017	Revision #	
Regulation Number	17	Required by:	<input type="checkbox"/> OHRP <input type="checkbox"/> Funding Agency <input type="checkbox"/> OLAW		
Applicability	<input type="checkbox"/> RGC Internal		<input checked="" type="checkbox"/> Researcher		<input type="checkbox"/> Institutional
Subgroup	<input type="checkbox"/> NKU Compliance	<input type="checkbox"/> IRB	<input checked="" type="checkbox"/> IACUC		<input type="checkbox"/> IBC

1.0 PURPOSE

The purpose of these guidelines is to describe acceptable methods for the euthanasia of animals used in teaching, research and testing at Northern Kentucky University. All animal euthanasia must be performed by appropriately trained personnel approved on the Animal Protocol.

2.0 GENERAL INFORMATION AND SCOPE

A. PERFORMANCE OF EUTHANASIA

All animal euthanasia must be performed by appropriately trained personnel approved on the Animal Protocol. All euthanasia procedures must be continuously monitored by the person(s) performing the procedure, until confirmation of euthanasia is complete.

B. CONFIRMATION OF EUTHANASIA

Any animal euthanized on a Northern Kentucky University IACUC protocol requires a method of confirmation of euthanasia. Some acceptable methods of confirmation are described below.

3.0 DETAILS

Common Acceptable Methods of Euthanasia

Listed below are some commonly used and accepted methods of euthanasia for different species. This list is not inclusive. Please see the "AVMA Guidelines on Euthanasia" for further information.

A. RODENTS WEIGHING > 500GRAMS

- Acceptable Methods of Euthanasia
 - Overdose of chemical anesthetics (typically 2-3 times the anesthetic dose)
 - Overdose of isoflurane (see "Isoflurane Euthanasia" below)
 - CO2 exposure (see the "Rodent CO2 Euthanasia" below)
- Barbiturate overdose
- Methods of Confirmation of Euthanasia
 - Bilateral thoracotomy
 - Decapitation
 - Vital tissue harvest (inclusive of heart and/or lungs and/or brain)

B. RODENTS WEIGHING <500 GRAMS

- Adults and neonates > 10 days of age
 - Acceptable Methods of Euthanasia
 - Overdose of chemical anesthetics (typically 2-3 times the anesthetic dose)
 - Overdose of isoflurane (see "Isoflurane Euthanasia" below)
 - CO2 exposure (please see the "Rodent CO2 Euthanasia" below)
 - Barbiturate overdose
 - Focused microwave irradiation
 - Methods of Confirmation of Euthanasia
 - Cervical dislocation (not acceptable for rats > 200 grams of body weight or hamsters due to their heavy cervical musculature)
 - Decapitation
 - Bilateral thoracotomy
 - Vital tissue harvest (inclusive of heart and/or lungs and/or brain)
 - Continued exposure to CO2 for at least 15 minutes after respiratory arrest

C. GUINEA PIG NEONATES:

Follow above guidelines for adults and neonates > 10 days

D. MOUSE, RAT AND HAMSTER NEONATES < 10 DAYS OF AGE

- Acceptable Methods of Euthanasia
 - Overdose of chemical anesthetics (typically 2-3 times the anesthetic dose)
 - Decapitation
 - NOTE: you do not have to use CO2 first. Per NIH guidelines, decapitation alone for this age group is an acceptable means of euthanasia, no confirmation required
- Methods of Confirmation of Euthanasia
 - Decapitation

E. FETI (UNBORN ANIMALS THAT HAVE NOT BREATHED)

- Mouse, Rat and Hamster Feti up to 15 days' gestation and Guinea Pig Feti up to 34 days' gestation:
 - Acceptable Methods of Euthanasia
 - Confirmed euthanasia of mother
 - Removal of feti from the anesthetized mother
 - No further method of confirmation of euthanasia of the feti required
- Mouse, Rat and Hamster Feti 15 days of gestation to birth and Guinea Pig Feti 35 days gestation to birth:
 - Acceptable methods of Euthanasia
 - Decapitation with scissors
 - Confirmed euthanasia of mother (feti not required for study)
 - Confirmed euthanasia of mother (feti required for study)

- The uterus with the pups or the pups with the amniotic can be removed after euthanasia of the mother
- If at any point a fetus is allowed to breathe it must be decapitated
- Rapid freezing of feti while anesthetized (liquid nitrogen immersion)
 - 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 breathe it must be decapitated
- Methods of confirmation of euthanasia
 - No further method of confirmation of euthanasia of the feti required
 - If the mother is euthanized, her death must be confirmed

F. RODENT CO2 EUTHANASIA

- Animals must not be combined from different cages
- If euthanizing an entire cage the animals must remain in their original housing. If euthanizing part of the cage, move to a clean cage with a filter top.
- The maximum number of mice per cage is 5 mice.
 - Exception: breeder pair with their unweaned litter
- Flow Rate Instructions for Rodent Co2 Euthanasia
 - Adjust CO2 flow rate to a minimum of 30%.
 - Continue CO2 until one minute after breathing stops
 - Confirm Euthanasia as described previously in this document
- See the NKU IACUC SOP #11 Rodent Cage Regulations for standard cage sizes

G. ISOFLURANE EUTHANASIA

- Adjust the isofurane flow rate or concentration to 5% or greater
- Continue isoflurane exposure until one minute after breathing stops

Confirm Euthanasia as described previously in this document

H. XENOPUS

- Acceptable Methods of Euthanasia
 - Tricaine Methane Sulfonate(TMS, MS 222) 5-10 grams/L buffered with sodium bicarbonate for a pH between 7-7.5
 - Barbiturate overdose
 - Decapitation or cervical sectioning while anesthetized
 - Euthanasia must be confirmed by double pithing
- Methods of Confirmation of Euthanasia
 - Bilateral thoracotomy
 - Double pithing
 - Sternotomy
 - Vital tissue harvest (inclusive of heart and/or lungs and/or brain)
 - Decapitation or cervical section

I. ZEBRAFISH (8 DAYS POST FERTILIZATION (DPF) AND OLDER)

- Acceptable Methods of Euthanasia
 - Tricaine Methane Sulfonate (TMS, MS 222) \geq 250mg/L buffered with sodium bicarbonate for a pH between 7-7.5
 - Barbiturate overdose
 - Rapid chilling: Submerge fish in 2-4°C chilled water
 - Fish should not be in direct contact with ice
 - Fish must remain in the chilled water for 30 minutes following cessation of opercular movement
 - Immersion in CO₂ saturated water (NOTE: some fish may exhibit hyperactivity with this method)
- Methods of Confirmation of Euthanasia
 - Observation of no opercular movements for at least 30 minutes following anesthetic overdose or chilling
 - Decapitation

J. ZEBRAFISH 4-7 DPF

- Acceptable Methods of Euthanasia
 - Tricaine Methane Sulfonate (TMS, MS 222) \geq 250mg/L buffered with sodium bicarbonate for a pH between 7-7.5
 - Rapid chilling: Submerge fish in 2-4°C chilled water
 - Fish should not be in direct contact with ice
 - Fish must remain in the chilled water for 30 minutes following cessation of opercular movement
 - Immersion in CO₂ saturated water (NOTE: some fish may exhibit hyperactivity with this method)
 - Exposure to a dilute (1-10%) sodium hypochlorite or calcium hypochlorite solution
- Methods of Confirmation of Euthanasia
 - Observation of no opercular movements for at least 30 minutes following anesthetic overdose or chilling
 - Decapitation

K. ZEBRAFISH FRY 0-3DPF

- Acceptable Methods of Euthanasia
 - Exposure to a dilute (1-10%) sodium hypochlorite or calcium hypochlorite solution
 - Two step euthanasia:
 - 1. MS222 or rapid chilling as described above
 - 2. Exposure to a dilute sodium hypochlorite or calcium hypochlorite solution
- Methods of Confirmation of Euthanasia
 - The exposure to a dilute sodium hypochlorite or calcium hypochlorite solution confirms euthanasia

L. NON-RODENT MAMMALS

- Acceptable Methods of Euthanasia (AVMA guidelines)
 - Overdose of chemical anesthetics (typically 2-3 times the anesthetic dose)
 - Overdose of isoflurane (see “Isoflurane Euthanasia” above)
 - Barbiturate overdose
- Methods of Confirmation of Euthanasia
 - Bilateral thoracotomy
 - Sternotomy
 - Vital tissue harvest (inclusive of heart, lungs, and/or brain)

M. FISH, REPTILE, AND AMPHIBIAN

- Acceptable Methods of Euthanasia
 - Tricaine Methane Sulfonate (TMS, MS 222) \geq 250mg/L buffered with sodium bicarbonate for a pH between 7-7.5
 - Barbiturate overdose
 - Rapid chilling: Submerge fish in 2-4°C chilled water
 - Fish should not be in direct contact with ice
 - Fish must remain in the chilled water for 30 minutes following cessation of opercular movement
 - Immersion in CO₂ saturated water (NOTE: some fish may exhibit hyperactivity with this method)
- Methods of Confirmation of Euthanasia
 - Observation of no opercular movements for at least 30 minutes following anesthetic overdose or chilling
 - Decapitation

N. EUTHANASIA BY PERFUSION

- Rodents
 - Any animal undergoing perfusion must be under a surgical plane of anesthesia before any incisions are made. A surgical plane of anesthesia must be maintained until the heart stops.
 - Any person performing euthanasia by perfusion must be properly trained.
- Non-Rodents
 - Any animal undergoing perfusion must be under a surgical plane of anesthesia before any incisions are made. A surgical plane of anesthesia must be maintained until the heart stops.
 - Any person performing euthanasia by perfusion must be properly trained.

O. PHYSICAL METHODS OF EUTHANASIA ON UN-ANESTHETIZED ANIMALS > 10 DAYS OLD

- Physical methods of euthanasia on an un-anesthetized animal in an Animal Protocol must be justified and approved by the IACUC.
- The IACUC Compliance and Training Coordinator must observe one euthanasia without anesthesia performed by the person responsible for training lab personnel.

- Any person performing a physical method of euthanasia (i.e. decapitation, cervical dislocation) on an un-anesthetized animal must be properly trained.

4.0 REFERENCES

"AVMA Guidelines for the Euthanasia of Animals: 2013 Edition" AVMA.org., 2013.

[Guide for the Care and Use of Laboratory Animals Eighth Edition](#)

5.0 FORMS OR ATTACHMENTS

6.0 DEFINITIONS

Approvals

Title	Approved	Date Approved	Not Applicable
Manager of Research Compliance	<input checked="" type="checkbox"/>	06/13/2018	<input type="checkbox"/>
IACUC Chair	<input checked="" type="checkbox"/>	06/13/2018	<input type="checkbox"/>
Institutional Official	<input type="checkbox"/>		<input checked="" type="checkbox"/>

Revisions

Title	Approved	Date Approved	N/A	Summary
Manager of Research Compliance	<input checked="" type="checkbox"/>	12/14/2021	<input type="checkbox"/>	Revised due based Co2 limits based on AVMA requirements
IACUC Chair	<input checked="" type="checkbox"/>	12/14/2021	<input type="checkbox"/>	
Institutional Official	<input type="checkbox"/>		<input type="checkbox"/>	