SAN DIEGO STATE UNIVERSITY

IACUC Guidelines

Guidelines on Euthanasia for Animals used in Research and Teaching

IACUC Approved: March 9, 2020

SDSU Guidelines on Euthanasia

The PHS Policy requires that euthanasia be conducted in a manner consistent with the American Veterinarian Medical Association (AVMA) Guidelines for Euthanasia: 2020 Edition, as well as the National Research Council's *Guide for the Care and Use of Laboratory Animals* (Eighth Edition) (OLAW). The San Diego State University IACUC has approved these Guidelines and Standard Operating Procedures to assist the teaching and research community in applying the regulatory requirements, and to promote best practices in euthanasia of laboratory animals.

Definitions

Distress: is the effect of stimuli that initiate adaptive responses that are not beneficial to the animal – thus, the animal's response to stimuli interferes with its welfare and comfort.

Euthanasia: derived from the Greek terms *eu* meaning "good" and *thanatos* meaning "death", is the act of providing humane death in animals, by methods that induce rapid unconsciousness and death with minimal pain, distress and anxiety. Euthanasia may be planned and necessary at the end of a protocol, or it may be a means to end pain and distress that cannot be alleviated by other means. The criteria involved in successful euthanasia include, a rapid loss of consciousness followed by cardiac or respiratory arrest, and the loss of brain function. The method of euthanasia should minimize the distress and anxiety experienced by the animal prior to loss of consciousness, and is determined by the following factors: species, age, weight, health status, skill of personnel, number of animals, and compatibility with the scientific requirements of the study.

Pain: is a sensation (perception) that results from nociceptive nerve impulses reaching areas of the brain capable of conscious perception via ascending neural pathways.

Unconsciousness: is the loss of individual awareness that occurs when the brain's ability to integrate information is blocked or disrupted. The onset of unconsciousness in animals is associated with loss of the righting reflex.

Considerations

The method of euthanasia used must be approved in your IACUC protocol. Euthanasia must be done in the most humane manner possible for individual species, within the scientific limits of the study. Species may perceive pain and distress, and express behaviors associated with pain and distress, in a variety of ways, therefore methods of euthanasia should be employed that take into consideration species specific

physiology, anatomy, and behavior. When performing euthanasia, it is necessary to acknowledge the human-animal bond. Attachments can occur in the research setting that may cause personnel performing euthanasia to experience stress or grief. To minimize this effect, it is important that personnel fully understand the euthanasia process. Euthanasia must be conducted by personnel appropriately trained in the method, and to recognize cessation of vital signs in the species being euthanized. Death must be confirmed before disposal of the animal remains.

Conduct

1. It is the responsibility of the individual performing euthanasia to assure that the animal intended for euthanasia is the correct animal. In cases where OLAC staff has been asked to perform euthanasia, it is the responsibility of lab staff to appropriately label the cages of animals to be euthanized.
2. When euthanasia as a means of alleviating animal suffering is recommended by veterinary staff, lab staff must respond in a timely manner.
3. Animals should be euthanized out of the sensory range of other animals, particularly conspecifics, whenever possible.
4. In animals that are not accustomed to being handled, stress can be reduced by minimizing visual, auditory, and tactile stimulation.
5. When using inhalants like CO_2 for euthanasia, animals should be euthanized without disruption of social groups or removal from the home cage whenever possible. Only conspecifics and compatible cohorts should be euthanized together, and euthanasia chambers must not be overcrowded. Do not combine cages of males as the addition of strange males to the cage causes stress due to unfamiliar pheromones and can lead to fighting.
6. In all cases death must be confirmed by one of the following:
    a. Decapitation (or cervical dislocation)
    b. Bilateral thoracotomy
    c. Vital tissue harvest (including heart, and/or lungs, and/or brain)
    d. Exsanguination

Common Methods for Laboratory Rodents (not all-inclusive, please refer to the AVMA Guidelines for Euthanasia: 2013 Edition for more information)

Methods are categorized as acceptable, acceptable with conditions and unacceptable according to the American Veterinary Medical Association Guidelines for Euthanasia of Animals: 2013 edition. **Acceptable** methods are those that consistently produce a humane death when used as the sole means of euthanasia. **Acceptable with conditions**, are methods that by the nature of the technique or because of greater potential for operator or safety hazards might not consistently produce humane death, or are methods not well documented in scientific literature. **Unacceptable** methods are those techniques that are deemed inhumane under any conditions or pose a substantial risk to the person applying the technique. There are also **adjunctive** methods, which are methods that cannot be used as the sole method of euthanasia, but can be used in conjunction with other methods to produce a humane death.

Acceptable Methods

1. **Barbiturate overdose** - Overdosing with a commercial injectable euthanasia solution or injectable pharmaceutical grade sodium pentobarbital (100 mg/kg) can be used to euthanize all animal species and is appropriate for all ages. The recommended dosage for euthanasia is found in the product label information. Because these are controlled substances, they must be procured with a DEA license. Many anesthetics/analgesics are DEA regulated. The PI must contact EH&S for guidance. These preparations can be administered intravenously or intraperitoneally. Do not administer these compounds subcutaneously or intramuscularly.
2. **Carbon Dioxide (CO_2) overdose** – Only compressed CO_2 gas in cylinders with a flow regulator may be the source of CO_2 as precise flow regulation is necessary to reduce pain and distress associated with rapid filling of the euthanasia chamber with CO_2. The chamber must be set to receive CO_2 at a rate of 30-70% displacement of room air per minute but **NOT** pre-filled. It is recommended to use 30% flow rate until the animals are unconscious then turn it up to 70% or above. Death must be confirmed by an adjunctive method. The chamber must be turned upside down to discard accumulated CO_2 and disinfected after

each use. This method can be used on all rodents excluding neonates. Neonatal rodents <10 days of age are resistant to hypoxia and may require exposure of 30 minutes or more to CO_2.
3. **Cervical Dislocation** - This procedure is only for use in rodents under 200 grams body weight. This method is humane when applied by individuals with a demonstrated high degree of technical proficiency. Campus Veterinarians or OLAC Manager may certify individuals demonstrated technical proficiency. In lieu of demonstrated technical competence, animals must be sedated or anesthetized prior to cervical dislocation.
4. **Inhalant Anesthetic overdose -** The animal(s) are placed in a closed chamber containing cotton or gauze soaked with an appropriate amount of anesthetic, or placed in an induction box or nose cone attached to an anesthetic machine with the vaporizer set at 5%. Death must be confirmed by an adjunctive method or continued exposure to the anesthetic vapors for at least 5 minutes after respiratory arrest. The liquid state of most anesthetics is irritating therefore animals should be prevented from contact with the cotton or gauze in the chamber. Inhalant anesthetic exposure is considered a human health hazard. All inhalation anesthetics must be used under a fume hood or with approved gas scavenging equipment. Inhalation anesthetics for euthanasia are listed in order of preference: isoflurane, sevoflurane.

Acceptable with Conditions
1. Decapitation - This method can be used for rodents and allows for the recovery of tissues and body fluids that are chemically uncontaminated as well as anatomically undamaged brain tissue. Decapitation without sedation or anesthetic must be scientifically justified, unless being performed on neonatal rodents <10 days old. Plastic restraint cones are recommended when decapitating rodents to reduce the stress that accompanies the restraint in conscious animals. Decapitation requires the use of specially designed equipment (guillotines for adults or scissors for neonates) that must be maintained to ensure the sharpness of the blades. The operator must be trained in the use of the guillotine, and the handling and restraint of the animals when performing this technique. For neonatal rodents <10 days of age, this is the preferred method of euthanasia. Other rodents should not be in the room during decapitation.

Adjunctive Methods
When using techniques other than cervical dislocation, decapitation, or barbiturate overdose, an adjunct method should be included to assure death and prevent resuscitation. Approved adjunctive methods are listed below.
1. **Cervical Dislocation** - See as described under Approved Methods.
2. **Exsanguination** - Death can be assured by the removal of a large volume of blood. This technique is never performed on a conscious animal. Animals may be exsanguinated to obtain blood products, but only when they are sedated or anesthetized.
3. **Pneumothorax** - To create a pneumothorax on an anesthetized or unconscious animal, a cut is made bilaterally through the chest wall using scissors or a scalpel blade or the diaphragm can be lacerated. The heart can also be cut or removed to ensure death.
4. **Decapitation** - See as described under Approved Methods.

Common Methods for Fish (not all-inclusive, please refer to the AVMA Guidelines for Euthanasia: 2020 Edition for more information)

Descriptions of methods used to euthanize fish follow and include 1-step and 2-step procedures following immersion in anesthetic solutions to assure death. Fish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement.

1. MS 222 (tricaine methanesulfonate) - Fish are placed in a tank with >250 mg/L MSS222 with the pH neutralized using sodium bicarbonate. The fish should be immersed for 30minutes. Due to species differences in response to MS 222, a secondary method of euthanasia such as decapitation followed is recommended to assure death.
A stock solution of MS 222 can be prepared as follows: 10g/L MS 222 neutralized with sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5. The solution should be protected from light and refrigerated or frozen. The solution should be replaced monthly or any time a brown color is observed. Occupational exposure to MS 222 has been associated with retinal toxicity in humans.

2. Benzocaine or benzocaine hydrochloride - Benzocaine or benzocaine hydrochloride, solutions should be prepared in concentrations > 250 mg/L and must be buffered. The fish should be immersed for 30minutes. Due to species difference in response to benzocaine, a secondary method of euthanasia is recommended to assure death. An acceptable secondary method of euthanasia is decapitation or opening of the body cavity.
Benzocaine base is not water soluble and must be prepared as a stock solution at 100g/liter in acetone or ethanol. Benzocaine-containing products should be protected from light and protected from freezing or excessive heat over 40 degrees C.
3. Physical disruption of brain activity – Physical disruption of brain activity is considered humane and painless if performed properly. Physical disruption can be produced through a blow to the skull resulting in concussive stunning followed by physical destruction of the brain using a pithing rod. Euthanasia must be executed by a skilled operator to ensure a quick and painless death.

Common Methods for Reptiles (not all-inclusive, please refer to the AVMA Guidelines for Euthanasia: 2013 Edition for more information)

1. Pentobarbital-containing solution (pharmaceutical grade) injected intra-abdominally or, in case of a fractious animal, put into the mouth. It is recommended that the initial methods of euthanasia be followed with a second technique to ensure non-recovery. These additional methods include opening the thoracic or abdominal cavity and cutting a major vessel, other methods of exsanguination, or perfusion. For all secondary methods, adequate anesthetic depth must be ensured prior to performing procedure.

2. MS 222
Snakes Individuals will be euthanized through an injection of MS 222 into the coelomic cavity while restrained in a snake tube. This procedure follows the MS 222 (Tricaine methanesulfonate) procedure laid out by Conroy et al. 2009 ("Use of Tricaine Methanesulfonate for Euthanasia of Reptiles", J. Am. Assc. Lab. Anim. Sci. 47(5)) at the Office of Laboratory Animal Care at U.C. Berkeley. This protocol involves two injections of MS 222 into the coelomic cavity; the first injection is 250-500mg/kg of 1% sodium bicarbonate-buffered MS-222 followed shortly thereafter by an injection of 1.0mL unbuffered 50% MS222. The authors found that this procedure was an acceptable replacement to injections of sodium pentobarbital in reptiles.
A stock solution of MS 222 can be prepared as follows: 10g/L MS 222 neutralized with sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5. The solution should be protected from light and refrigerated or frozen. The solution should be replaced monthly or any time a brown color is observed. Occupational exposure to MS 222 has been associated with retinal toxicity in humans.

3. Overdose of isoflurane- Overdose of isoflurane using open drop technique. After euthanasia, animals will be monitored for 3 minutes to ensure absence of movement, heartbeat and respiration and no response to pinch reflex. Cervical dislocation will then be administered to assure that they do not recover from anesthesia overdose.

Lizards Lizard specimens to be vouchered will be euthanized following a procedure newly described ("Use of Tricaine Methanesulfonate for Euthansia of Reptiles"; J. Am. Assc. Lab. Anim. Sci., 2008, volume 47, #5) by an individual at the Office of Laboratory Animal Care (UC Berkeley). The researcher concluded that this method was an acceptable alternative to sodium pentobarbital injection. This protocol is a 2-step approach involving the injection of Tricaine methanesulfonate (MS-222) into the coelomic cavity. The 1st step involves the intracoelomic (ICm) injection of 250-500 mg/kg of 1% sodium bicarbonate buffered MS-222. After a few minutes (specimens show no response to toe or skin pinch), a 2nd ICm injection of 0.1-1.0 ml of unbuffered 50% MS-222 is administered.

Common Methods for Amphibians (not all-inclusive, please refer to the AVMA Guidelines for Euthanasia: 2013 Edition for more information)

Frogs:

1. Exposure to isoflurane via gel mixture - as per Setter (2007). Isoflurane anesthetic is made: 1.5ml water + 3.0 ml isoflurane + 3.5ml K-Y jelly. Dosage depends on body mass (0.025-0.035 ml/g body mass). This mixture creates a thick liquid, rather than a gel as described by Setter (2007). A 2 ml mixture is applied. This volume may begin to run off the back so it may be necessary to apply some also to the ventral surface. Euthanasia can also be achieved through inhalation of isoflurane in a precision vaporizer.

2. Use of tricaine methanesulfonate (MS-222) - A concentration solution of at least 250 mg/L is recommended for euthanasia; a concentration of 1 g/L has been shown to be effective. MS 222 is acidic and should be buffered with sodium bicarbonate to saturation (pH 7.0 - 7.5). The animal should be left in the solution for at least 30 minutes following cessation of respiratory movements. A secondary method of euthanasia is necessary to assure death.
*Methods to ensure death can include decapitation followed by double pithing, or opening of the body cavity and removal of the heart.
A stock solution of MS 222 can be prepared as follows: 10g/L MS 222 is neutralized with sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5. The solution should be protected from light and refrigerated or frozen. The solution should be replaced monthly or any time a brown color is observed. Occupational exposure to MS 222 has been associated with retinal toxicity in humans.

3. Benzocaine hydrochloride - Use of 100-200mg/liter of water or a topical application of 7.5% or 20% benzocaine hydrochloride gel on the amphibian's ventrum. Benzocaine base is not water soluble and must be prepared as a stock solution at 100g/liter in acetone or ethanol. Benzocaine-containing products should be protected from light and protected from freezing or excessive heat over 40 degrees C. A secondary method of euthanasia is necessary to assure death.
*Methods to ensure death can include decapitation followed by double pithing, or opening of the body

Common Methods for Birds (not all-inclusive, please refer to the AVMA Guidelines for Euthanasia: 2013 Edition for more information)

Chickens

1. Overdose of inhalant anesthetic - Inhaled anesthetics may be used at high concentrations as a sole method of euthanasia or may be used to render birds unconscious prior to application of other methods of euthanasia. Isoflurane inhalation may be used to effect.

2. Pentobarbital overdose - Pentobarbital overdose at 100 mg/kg iv is an effective method of euthanasia. Pharamceutical grade solutions, such as commercial euthanasia solutions, must be used.

3. Decapitation – Decapitation is acceptable with conditions for the euthanasia of poultry when performed by competent personnel. Decapitation should be executed with a sharp instrument, ensuring rapid and unobstructed severing of the head from the neck. Use of a bleeding cone may facilitate restraint.

Wild Bird Species

A primary factor influencing methods selected for euthanasia of free-ranging birds is lack of control over the animal. Given that close human contact is difficult to achieve these animals may have to be euthanized or immobilized from a distance.

1. Shotgun by trained personnel - Due to the lack of control over free-ranging wildlife, firearms may be the most appropriate approach to their euthanasia. A properly placed gunshot can cause immediate insensibility and humane death. Shooting should only be performed by highly skilled personnel trained in the use of firearms. The preferred target area should be the head. A gunshot to the heart or neck does

not immediately render animals unconscious, but may be required when it is not possible to meet the Panel on Euthanasia definition of euthanasia.

If wildlife can be captured, the following euthanasia methods are acceptable.

2. Euthanasia Solution IV Pharmaceutical grade Pentobarbital overdose at 100-150 mg/kg iv is an effective method of euthanasia. Smaller species with higher metabolic rates may require the higher dose where larger birds may be use the lower dose. Wild, fearful, or excited birds may require a sedative or anesthesia before IV injections can be performed. When IV injection is impossible, injectable euthanasia agents can be administered via intracoelomic, intracardiac, or intraosseous routes only if a bird is unconscious or anesthetized. Injection into air sacs must be avoided due to delayed absorption, irritation and respiratory compromise. Euthanasia agents should also not be administered into the humerus or femur due to potential for irritation to the repiratory system or drowning.

3. Cervical dislocation - Cervical dislocation can be used for small birds (<200g) when no other method is available, but the procedure has been performed on birds as large as 2.3kg (5.1lb). It should only be performed by well-trained personnel who are monitored to ensure proficiency. A secondary method such as decapitation or exsanguination should be employed to ensure death when feasible.