

Taipei Medical University Institutional Animal Care and Use Committee Early Termination of Experiments and Euthanasia Regulations for Laboratory Animals

September 23, 2016 version (1st revision)

I. Legal basis:

According to Article 17 Chapter 3 of the Animal Protection Act, “one shall examine the conditions of a lab animal immediately after a scientific application is completed. Animals whose quality of life has been compromised from losing limbs or organs or from pain shall be immediately put to death in the least painful way.”

II. Timing of euthanasia procedure: except for cases that are approved by the Institutional Animal Care and Use Committee (IACUC) (e.g., symptoms caused by the experiment can be anticipated and the pain caused by these symptoms should be minimized), the researcher must stop the experiment immediately or proceed with euthanasia on all animals during the experiment or animals that have not undergone the experiment if one of the following conditions is encountered.

1. Weight loss: when an animal loses 20% of its original weight (including the stop of weight gain in an animal during its growth period) or loses more than 15% of its weight within 72 hours following an experiment. Alternatively, the presentation of cachexia or wasting syndromes in an animal. For weight loss in an animal undergoing a non-growth period, its weight when it first entered the laboratory or its average weight depending on its age can be used as bases. An animal in its growth period may not experience weight loss, but if it fails to gain weight, it should be considered as weight loss.
2. Depraved appetite: no food intake for 24 hours for small rodents; no food intake for 5 days for middle- and large-sized animals. Alternatively, loss of appetite (less than 50% of the usual food intake) for 3 days in small rodents and for 7 days in middle- and large-sized animals.
3. Debilitating/dying status: an animal is clearly debilitating even without being anesthetized or tranquilized; when an animal fails to stand or can barely stand, and the situation persists for

24 hours; or when it shows little or no response to external stimulation and experiences a body temperature drop.

4. Infection: a clearly perception or increase in white blood cell count and body temperature increase that are judged to be caused by infection; this increase has to occur when the antibiotic treatment is ineffective and be accompanied by systemic discomfort in animals.
5. When clinical symptoms severely impairing the function of organs occur and the treatment of these symptoms is ineffective; alternatively, when a veterinarian judges that the animal's prognosis is poor.

5.1. Respiratory system status: dyspnea and cyanosis.

5.2. Cardiovascular system status: substantial blood loss and anemia remaining even after a transfusion treatment was carried out (packed cell volume lower than 20%).

5.3. Digestive system status: severe vomiting or diarrhea, gastrointestinal obstruction, intussusception, peritonitis, and organ ablation.

5.4. Urinary system status: renal failure (increase in blood urea nitrogen, creatinine, and uroperitoneum).

- 5.5. Nervous system status: central nervous system suppression, tremor, and paralysis (of one of the limbs), and pain that is not alleviated by analgesics.
- 5.6. Musculoskeletal system status: muscle damage or fracture that leads to limb dysfunction (except for anticipated dysfunctions that are approved by the IACUC).
- 5.7. Skin: incurable wound, repetitive selfmutilation, or insulation pad burns that are second degree or above.
6. Tumor: growth of a tumor covering over 10% of the animal's original weight or growth of a tumor measuring more than 20 mm for mice and 40 mm for rat. Infection or tumor necrosis caused by tumor metastasis or the rapid growth of a tumor to its ulceration. When a fixed tumor exceeds 10% of the weight of rodents (1 cm = 1 gm) or when tumor growth hinders the ability of animals to eat, drink, and move.
7. Uncontrollable pain and discomfort: when an animal exhibits pain or discomfort that cannot be alleviated by analgesics or when a veterinarian judges that the animal is no longer suitable for further experiments.

III. Reference:

1. Guide for the Care and Use of Laboratory Animals (Council of Agriculture, June 22, 2018 version).
2. Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice. Lab Animal Science, Vol49, No.3, 319-323.

IV. Appendices:

Appendix 1: tumor-caused early experiment termination and clinical evaluation of tumor symptoms

Appendix 2: body condition scoring table for small rodents

Appendix 3: explanation of euthanasia methods through carbon dioxide for small laboratory animals and rodents

Appendix 4: agents and methods of euthanasia by species

Appendix 5: agents and methods that are unacceptable as a primary method of euthanasia

Appendix 1 tumor-caused early experiment termination and clinical evaluation of tumor symptoms

Evaluation item	Termination standard	Clinical symptom evaluation
Physiological state	Loss of more than 20% of an animal's original weight (including weight gain stopping during an animal's growth period) or loss of more than 15% of an animal's weight within 72 hours following an experiment. The percentage of animal weight loss is calculated as follows: (animal's current weight - tumor weight)/animal basic weight × 100%	Weight loss and debilitation
Tumor diameter	Mice: one side larger than 20 mm in length Rat: one side larger than 40 mm in length	Measure and record tumor size through a scale periodically
Tumor weight	Mice: tumor weight reaches 10% of the mouse's weight Rat: tumor weight reaches 5% of the rat's weight Tumor weight (mg) = tumor volume (mm ³) = [short side (mm) ² × long side (mm)]/2 Weight percentage = tumor weight* / animal basic weight × 100% *If more than one tumor exists, the weights of all tumors should be added together.	Measure and record tumor weight periodically
Tumor external appearance	Necrosis or septicemia	Ulceration, emphysema, and purulent secretions can be observed on the tumor's external

		appearance.
Tumor location	Physiological dysfunction	Unable to eat or drink or carry out normal physiological functions (e.g., walking, urination, and defecation).

Appendix 2 body condition scoring table for small rodents

BC 1 體態瘦弱	極度顯露出骨骼與脊椎節，幾乎無肌肉覆蓋。
BC 2 體態不良	脊椎節可見，觸摸可感受背部骨盆骨。
BC 3 體態良好	脊椎與骨盆不明顯，觸摸可感受肌肉彈性。
BC 4 體態過度	脊椎呈柱狀，觸摸肌肉感覺堅硬。
BC 5 體態過胖	小鼠體態光滑且龐大，骨骼系統包埋在肌肉和皮下脂肪中無法觸及。



BC 1

- Mouse is emaciated.
- Skeletal structure extremely prominent; little or no flesh cover.
 - Vertebrae distinctly segmented.



BC 2

- Mouse is underconditioned.
- Segmentation of vertebral column evident.
 - Dorsal pelvic bones are readily palpable.



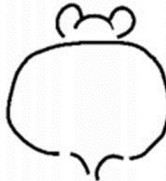
BC 3

- Mouse is well-conditioned.
- Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.



BC 4

- Mouse is overconditioned.
- Spine is a continuous column.
 - Vertebrae palpable only with firm pressure.



BC 5

- Mouse is obese.
- Mouse is smooth and bulky.
 - Bone structure disappears under flesh and subcutaneous fat.

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

Appendix 3 explanation of euthanasia methods through carbon dioxide for small laboratory animals and rodents (including mice, rats, hamsters, and guinea pigs)

Carbon dioxide inhalational anesthetics with or without presurgery are accepted euthanasia methods for small rodents by the American Veterinary Medical Association (AVMA). However, the following conditions must be met (reference to the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition):

1. High-pressure barreled carbon dioxide for commercial use or a gas tank must be used as the source of carbon dioxide.
2. Appropriate decompression adjustment and flow meter must be used to accurately regulate the flow of gas entering the euthanasia box.
3. When proceeding with euthanasia using carbon dioxide, the optimal flow rate of carbon dioxide should fill 10% to 30% of the volume of a euthanasia box per minute (cage volume/min).
4. Filling with carbon dioxide in advance and exposing an animal to 100% carbon dioxide is prohibited.
5. Adding oxygen to the carbon dioxide is not recommended.
6. If euthanasia cannot be conducted inside the cage where the animal was originally raised, another euthanasia box must be emptied and cleaned before it is used.
7. Confirming the death of an animal is necessary.

The carbon dioxide flow should be maintained for at least one minute after the animal has stopped breathing. An animal death can be confirmed by a physical examination. Alternatively, physically assisted euthanasia methods can be employed to confirm an animal's death. Additionally, calibration can be carried out by establishing euthanasia boxes and setting operational procedures. If the animal is not dead, a euthanasia method using carbon dioxide must be combined with another type of euthanasia method. Combining carbon dioxide with oxygen is currently considered to have no merit and can prolong the animal's dying time and might complicate judgement making.

Appendix 4 agents and methods of euthanasia by species

Species	Acceptable agents and methods	Agents and methods acceptable with conditions (an auxiliary method used after an animal is in a coma to ensure its death)
Teleost	Immersion (in buffered benzocaine 、 benzocaine hydrochloride 、 isoflurane 、 sevoflurane 、 quinaldine sulfate 、 buffered tricaine methanesulfonate 、 2- phenozyethanol), injected with pentobarbital, and rapid chilling	Decapitation, cervical transection, rapid chilling followed by maceration
Rodents	Injected with barbiturates and barbiturate combinations, dissociative agent combination	Inhalational anesthetics, carbon dioxide, carbon monoxide, ethanol, cervical dislocation, decapitation, and focused beam microwave irradiation
Rabbits	Intravenous barbiturates	Inhaled anesthetic overdose, carbon dioxide, cervical dislocation (as anatomically appropriate), penetrating captive bolt
Dogs	Intravenous barbiturates and injection of anesthetic to overdose	Barbiturates (alternative routes of administration), inhaled anesthetic excess, carbon dioxide, carbon monoxide, gunshots
Pigs	Injected with barbiturates	Carbon dioxide, carbon monoxide, nitrogen, argon, gunshots, electric shock, nonpenetrating captive blot, and manually applied blunt force trauma to the head
Poultry	Injected with barbiturates or death by anesthetic overdose	Carbon dioxide, carbon monoxide, nitrogen, argon, cervical dislocation (as anatomically appropriate), decapitation, gunshot, electrocution, captive blot, and manually applied blunt force trauma to the head

Appendix 5 agents and methods that are unacceptable as a primary method of euthanasia

Method	Explanation
Physical properties	
Air embolism	Can cause sputum, opisthotonus, and moaning in animals. This method can only be used on an anesthetized animal.
Stun/Blow to the head	An inhumane method that should not be used.
Burning	Burning by chemicals or heat are inhumane and should not be used.
Decompression	1. It causes pain to the animal and prolongs its dying time. 2. A young animal is resistant to the hypoxic state and requires a longer time to stop breathing. 3. This method can easily engender awakening in an animal. 4. This method engenders bleeding, vomiting, paralysis, and incontinence in animals.
Drowning	An inhumane method that should not be used.
Exsanguination	Substantial blood loss in an animal makes the animal anxious and angry. This method can only be applied to an animal that has lost consciousness.
Hypothermia/Rapid freezing	This method can only be applied to an animal that has lost consciousness. It can be used independently except for reptiles, amphibians, and rodents that were born within 5 days.
Suffocation/smothering	An inhumane method that should not be used.
Manually applied blunt force trauma to the head	An unusual euthanasia method that should be replaced by other euthanasia methods except for piglets and small animals.
Nonpenetrating captive bolt	An unusual euthanasia method. A pneumatic and nonpenetrating captive bolt is designed for special purposes. Specifically, the bolt is used on weaned piglets and newly born ruminants or turkeys.
Thoracic compression	Must not be implemented on an animal that is still conscious.

Chemical agents	
Chloral hydrate	Can cause irritation and respiratory depression in an animal and should not be used on any animal.
Chloroform	Is hepatotoxic and carcinogenic and should not be used.
Cyanide	Is harmful to human health and should not be used.
Diethyl ether	Can cause irritation and is inflammable and explosive; should not be used.
Formaldehyde	Immersing an animal in formaldehyde directly is inhumane, and this method should not be used on any species except spongiaria.
Neuromuscular blocking agents such as MgSO ₄ , KCl, and all curariform agents.	Not euthanasia agents and cause respiratory depression in the animal before it loses consciousness. Should not be used in an animal that is conscious.
Strychnine	Can cause severe spasms and muscle pumping in an animal and should not be used.
Reference	<ol style="list-style-type: none"> 1. The Council of Agriculture's announcement of: "euthanasia methods and anesthesia that are suitable or prohibited for vertebrate": prohibited methods to cause death of vertebrate . 2. AVMA Guidelines for Euthanasia of Animals: 2013 Edition Appendix 3.