

實驗中動物安樂死時機及準則

背景 Background

實驗中動物安樂死時機及準則由IACUC制訂，主要在規範尚未完成實驗之動物發生實驗所引起或非預期症狀，而必須將動物安樂死之狀況。

These criteria have been established by the Institutional Animal Care and Use Committee, which recognizes that euthanasia is sometimes necessary prior to the scheduled end of a study, either because of unanticipated complications, or because of the protocol itself.

準則 Criteria

此準則適用於所有實驗中或未實驗之動物。除了IACUC已審查同意之情形（如：實驗引起之預期中症狀且能使動物痛苦程度減至最低），否則只要符合下列任一項情況即需將動物安樂死。

These criteria apply to all animals on experimental protocols, whether manipulated or not. Fulfillment of one criterion can constitute grounds for euthanasia. Exceptions are permitted only if approved by the Committee as part of the protocol review process (i.e. the clinical signs listed below are expected as part of the experiment and appropriate measures are taken to minimize pain or discomfort in the animals).

1. 體重減輕 **Weight loss:**

體重減輕達20-25%，或是動物出現惡病質或消耗性症候時。

*非生長期動物體重減輕可依據動物剛進動物房之體重或平均年齡體重為依據。

生長期之動物體重或許不會下降，但若無法正常增重，仍應判為體重減輕。

Weight loss of 20-25% (depending on attitude, weight recorded at time of arrival, and age: growing animals may not lose weight, but may not gain normally) or if not measured, characterized by cachexia and wasting syndrome.

2. 喪失食慾 **Inappetance:**

小型齧齒類動物完全喪失食慾達24小時或食慾不佳（低於正常量之50%）達3天時。

大動物完全喪失食慾達5天或食慾不佳（低於正常量之50%）達7天時。

Complete anorexia for 24 hours in small rodents, up to 5 days in large animals; partial anorexia (less than 50% of caloric requirement) for 3 days in small rodents,

7 days in large animals.

3. 虛弱（無法進食或飲水） Weakness/inability to obtain feed or water:

動物在沒有麻醉或鎮靜的狀態下，長達24小時無法站立或極度勉強才可站立時。

Inability or extreme reluctance to stand, that persists for 24 hours, assuming that the animal has recovered from anesthesia.

4. 垂死/瀕死 Moribund state:

動物在沒有麻醉或鎮靜的狀態下，表現精神抑鬱伴隨體溫過低（低於37°C）時。

Depression coupled with body temperature below 37°C,

5. 感染 Infection:

無論是明顯可知或因體溫升高白血球數目增加而判斷為感染所致，且在抗生素治療無效並伴隨動物全身性不適症狀出現時。

Infection involving any organ system (either overt, or indicated by increased body temperature or WBC parameters), which fails to respond to antibiotic therapy within an appropriate period of time and is accompanied by systemic signs of illness.

6. 出現器官嚴重喪失功能的臨床症狀且治療無效，或經動物中心獸醫師判斷預後不佳時。如：

Signs of severe organ system dysfunction and non-responsive to treatment, or with a poor prognosis as determined by a veterinarian:

(1) 呼吸系統：呼吸困難、發紺

Respiratory system: dyspnea, cyanosis.

(2) 心血管系統：大失血、已給予一次輸液治療後仍貧血（低於20%）

Cardiovascular system: blood loss or anemia.

(3) 消化系統：嚴重嘔吐或下痢，消化道阻塞，套疊，腹膜炎，內臟摘除手術

Gastrointestinal system: severe vomiting or diarrhea, obstruction, intussusception; peritonitis, evisceration(immediate euthanasia required).

(4) 泌尿道系統：腎衰竭（BUN, creatinine, uroperitoneum的提升）

Urogenital system: renal failure characterized by elevated BUN, creatinine or uroperitoneum.

- (5) 神經系統：中樞神經抑制、震顫、癱瘓（其中任一肢或以上）、對止痛劑治療無效之疼痛

Nervous system: CNS depression, seizures, paralysis of one or more extremities; pain unresponsive to analgesic therapy.

- (6) 肌肉骨骼系統：肌肉受損或骨折使肢體喪失功能（實驗預期發生並通過IACUC審核除外）

Musculoskeletal system: muscle damage or fracture resulting in inability to use the limb, unless anticipated as part of the study.

- (7) 皮膚：無法治癒之傷口、重複性自殘或二級以上之保溫墊燙傷

Integumentary system: Non-healing wounds, repeated self-trauma, second or third degree heating pad burns.

7. 腫瘤生成終點評估 **Tumorigenesis Endpoints Assessment**

無論自發性或是實驗接種的腫瘤，均應進行實驗終點評估。當動物身上發現腫瘤，每週應至少檢查兩次腫瘤生長情形，兩次檢查的間隔不可超過四天。只要符合下列任一項情況即需將動物安樂死。

Tumors may occur spontaneously, or be experimentally induced through transplantation or administration of carcinogens. Animals in which tumors are growing should be checked at least two times weekly, at intervals no greater than four days apart. The clinical signs listed below are expected as part of the experiment and appropriate measures are taken to minimize pain or discomfort in the animals.

- 1) 單一腫瘤的重量超過動物體重的 10%，或是成年小鼠腫瘤平均直徑超過 20mm，或是成年大鼠腫瘤平均直徑超過 40mm。

A tumor burden greater than 10% body weight, and in an adult mouse, a mean tumor diameter exceeding 20 mm or in an adult rat, a mean tumor diameter exceeding 40 mm.

- 2) 體表腫瘤：腫瘤表面出現潰瘍、壞死或是感染。

Superficial tumors : Tumors that ulcerate, become necrotic or infected.

- 3) 腹腔腫瘤：腹腔異常擴張、呼吸困難。

Ascitic tumors - abdominal distension, dyspnea.

- 4) 顱內腫瘤：神經症狀。

Intracranial tumors - neurological signs.

動物安樂死方法 Method of euthanasia of animals at the end of study

表 1. 齧齒動物安樂死方法

方法	懷孕>13 日胚胎	01-06 日齡	07-14 日齡	<200g	>200g	備註
二氧化碳	∅	∅	V	V	V	1. 使用高壓桶裝二氧化碳 2. 執行時，儘可能在遠離動物飼養房的非公開場所進行 3. 14 日齡以下動物，不建議單獨使用二氧化碳安樂死，需配合斷頭
Halothane	∅	∅	V	V	V	須具備汽化器等專用設備，並設計合適的可透視性密閉容器以易於觀察動物 大部分的吸入性藥劑對人體有害，需在通風良好場所執行 麻醉誘導期動物可能出現掙扎及焦慮 7 公斤以上不建議使用此方法。
Methoxyflurane						
Isoflurane						
Enflurane						
Sevoflurane						
Desflurane						
Pentobarbital 100-150mg/kg. IP, IV	∅	∅	V	V	V	1. 屬於第三級管制藥品，人員須先向衛生署申請使用許可 2. 人員須備有保定動物及注射的技術
麻醉後放血	∅	∅	∅	V	V	1. 放血不完整或心臟採血不完整時，動物可能會甦醒 2. 人員須備有相關技術
麻醉後斷頭	V	V	V	V	V	1. 人員須備有相關技術 2. 仔鼠需以銳利的外科剪刀斷頭，其他則使用斷頭台
低溫麻醉後斷頭	V	V	∅	∅	∅	將仔鼠放置標本杯（非 petri dish），浸入冰漿中約二十分鐘，以減少腦部活性及流血
麻醉後頸椎脫白	∅	∅	∅	V	□	人員須備有相關技術
麻醉後注射 KCl. 2meq/kg, IV	∅	∅	∅	V	V	人員須備有靜脈注射的技術
清醒中動物斷頭	V	V	□	□	□	1. 人員須備有相關技術 2. 仔鼠需以銳利的外科剪刀斷頭，其他使用斷頭台
清醒中動物頸椎脫白	∅	∅	∅	□	□	人員須備有相關技術
乙醚	∅	∅	□	□	□	乙醚具有易燃、易爆的性質，對呼吸到黏膜刺激性強，如因實驗需求而使用乙醚，則需全程在抽氣櫃內操作，動物屍體需置於防爆冰櫃內

V：推薦使用的方法。∅：不推薦使用的方法。□：經機構內動物實驗管理委員會同意後使用的方法。IP：腹腔注射。IV：靜脈注射。

表.2 100% CO₂ 安樂死時間參考標準

Age of mice	CO ₂ exposure Time	Remark
0-6 days	60 mins	一般需配合斷頸法合併使用
7-13 days	20 mins	一般需配合斷頸法合併使用
14-20 days	10 mins	
≥21 days	5 mins	

來源：Comp Med 55(3), 275-281, 2005

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(摘自國家實驗動物中心資料)