



由統計學觀點探討實驗動物之 合理用量

白璐

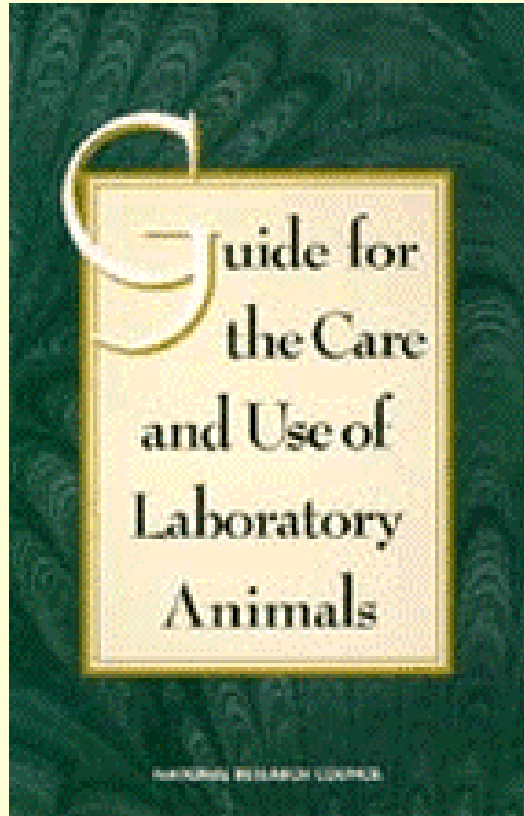
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例：某研究擬用大白鼠進行實驗，實驗共分**9**組，因考慮實驗過程中有些白鼠會因感染而死亡，故提出要使用**200**隻大白鼠

- 這樣的大白鼠數量要求合理嗎？
 - 研究者應提供哪些資訊？
- 

Guide for the Care and Use of Laboratory Animals



Institute of Laboratory Animal Resources
Commission on Life Sciences
National Research Council
National Academy Press
Washington, D.C.




MONITORING THE CARE AND USE OF ANIMALS

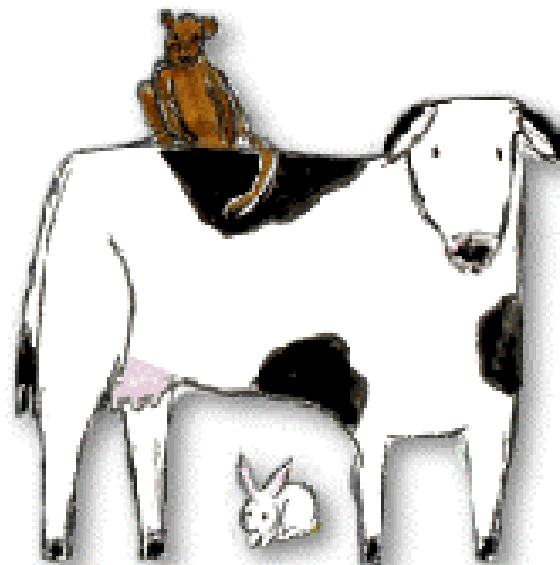
GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS

Animal Care and Use Protocols

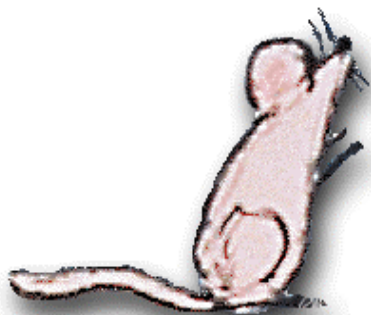
The following topics should be considered in the preparation and review of animal care and use protocols:

- Rationale and purpose of the proposed use of animals.
 - Justification of the species and number of animals requested. Whenever possible, the number of animals requested should be justified statistically.**
 - Availability or appropriateness of the use of less-invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see Appendix A, "Alternatives").
 - Adequacy of training and experience of personnel in the procedures used.
 - Unusual housing and husbandry requirements.
 - Appropriate sedation, analgesia, and anesthesia. (Scales of pain or invasiveness might aid in the preparation and review of protocols; see Appendix A, "Anesthesia, Pain and Surgery.")
 - Unnecessary duplication of experiments.
 - Conduct of multiple major operative procedures.
 - Criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated.
 - Postprocedure care.
 - Method of euthanasia or disposition of animal.
 - Safety of working environment for personnel.
- 

OLAW



SAMPLE DOCUMENTS FOR IMPLEMENTATION OF THE PHS POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS




Animal Study Proposal

- [View Document](#)
- [Download Document](#)
- [Instructions for Downloading](#)




VIEW THE DRAFT ANIMAL STUDY PROPOSAL

- Administrative Data
 - Animal Requirements
 - Transportation
 - Study Objectives
 - Rationale for Animal Use
 - Description of Experimental Design and Animal Procedures
 - Surgery
 - Pain or Distress Classification and Consideration of Alternatives
 - Anesthesia, Analgesia, Tranquilization, Other Agents
 - Methods of Euthanasia or Disposition of Animals at End of Study
 - Hazardous Agents
 - Biological Material/Animal Products for Use in Animals
 - Transgenic and Knockout Animals
 - Exemptions from Environmental Enhancement for Nonhuman Primates or Exercise for Dogs
 - Field Studies
 - Special Concerns or Requirements of the Study
 - Principal Investigator Certifications
 - Concurrences
 - Final Approval
 - Attachment 1 - Explanation for USDA Classification E
- 

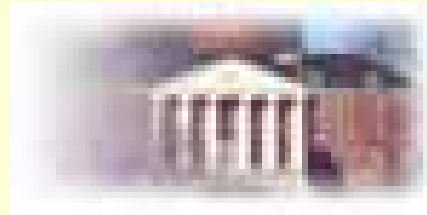


RATIONALE FOR ANIMAL USE

- 1) Explain your rationale for animal use. *[The rationale should include reasons why non-animal models cannot be used.]*
 - 2) Justify the appropriateness of the species selected. *[The species selected should be the lowest possible on the phylogenetic scale.]*
 - 3) Justify the number of animals to be used. *[The number of animals should be the minimum number required to obtain statistically valid results.]*
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NATIONAL INSTITUTES OF HEALTH
Office of Extramural Research



NIH Home


**Public Health Service Policy on Humane Care
and Use of Laboratory Animals**


OFFICE OF LABORATORY ANIMAL WELFARE

Amended August, 2002



U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training

- I. The transportation, care, and use of animals should be in accordance with the [Animal Welfare Act \(7 U.S.C. 2131 et. seq.\)](#) and other applicable Federal laws, guidelines, and policies.*
 - II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
 - **III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.**
 - IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
 - V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.
 - VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.
 - VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
 - VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.
 - IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.
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做動物實驗為何要求 用最少數量的動物

- 倫理的〔Ethical〕考量
- 經濟的〔Economic〕考量
- The “3Rs” principles

(Russell & Burch, 1959)

Replaced by less sentient alternatives


Refined to minimize any adverse
effects

Reduced to the minimum number





研究計畫內容的基本要求


- The objectives of the research &/or the hypotheses to be tested;
 - The reason for choosing their particular animal model;
 - The species, strain, source, and type of animal used;
 - The study design and the number of animals used; and
 - The statistical methods used for analysis.
- 



實驗動物用量的合理性

- 數量太少，實驗要看的效果無法顯現出來
- 數量過多，浪費了許多動物、金錢、時間、人力

合理量如何決定？



實驗研究的基本目的與 實驗動物用量的決定

- Pilot study [先驅研究]

無從決定實驗動物的合理用量

- Estimation [求出一個估計值]

與估計的準確性有關

- Testing hypothesis [檢定一個假說]

與檢定水準(α level)及檢力(power of the test)有關

實驗研究主要測量值的特性 與實驗動物用量的決定

- 連續性變項 (continuous variable)

 - 平均值 (mean, \bar{x})

 - 變異數 (variance, σ^2)

- 二分變項 (dichotomous variable)

 - 百分比 (proportion, p) (即平均值)

 - 變異數 (variance, pq/n) ($q=1-p$)

求估計值時決定動物用量的 先備資訊

- 該種測量值的變異數 (variance, σ^2)
或標準差 (standard deviation, σ)
- 估計的準確性之信心程度係數 (z,
Coefficient of a certain level of
Confidence Interval, e.g. $z=1.96$ for
95%CI)
- 估計的準確性 (Confidence Interval)
——與真值的相對差異 (d , $\frac{1}{2}$ CI)



先備資訊從何處獲得？

- 學理基礎
 - 文獻
 - Pilot study
 - 經驗印象
- 

估計平均值時實驗動物樣本數的估算

● $n \geq z^2 \sigma^2 / d^2$

e.g. 某測量值的變異數為0.64，在95%的信心程度，誤差不超過 ± 0.5 的條件下，需要多少隻實驗動物？

95%CI 之係數 $z=1.96$

$$n \geq (1.96)^2(0.64)/(0.5)^2 \geq 9.83 \doteq 10$$

- 
- 若要求99%的信心程度，則 $z=2.58$


$$n \geq (2.58)^2(0.64)/(0.5)^2 \geq 17.04 \doteq 18$$

- 若要求誤差不超過 ± 0.4 ，則

$$n \geq (1.96)^2(0.64)/(0.4)^2 \geq 15.37 \doteq 16$$

- 若變異數較大， $\sigma^2=1$ ，則

$$n \geq (1.96)^2(1)/(0.5)^2 \geq 15.37 \doteq 16$$

- 在合理的調整各假設值的情況下，實驗動物數量的估計不會超過原始估計數的兩倍
- 

估計百分比時實驗動物樣本數的估算

● $n \geq z^2 pq / d^2$

e.g. 估計動物對某種刺激的反應率為30%，
在95%信心程度與誤差不超過±10%的條件
下，需要多少隻實驗動物？

95%CI 之係數 $z=1.96$

$$n \geq (1.96)^2 (.3)(.7) / (0.1)^2 \geq 80.7 \doteq 81$$

- 
- 若要求99%的信心程度，則 $z=2.58$


$$n \geq (2.58)^2(0.21)/(0.1)^2 \geq 139.8 \div 140$$


- 若要求誤差不超過 $\pm 8\%$ ，則

$$n \geq (1.96)^2(0.64)/(0.08)^2 \geq 126.2 \div 127$$


- 若反應率增加為40%，則

$$n \geq (1.96)^2(.4)(.6)/(0.1)^2 \geq 92.2 \div 93$$

- 在合理的調整各假設值的情況下，實驗動物數量的估計仍不會超過原始估計數的兩倍
- 



檢定假說時決定動物用量的 先備資訊

- 該種測量值的變異數 (variance, σ^2)
或標準差 (standard deviation, σ)
 - 顯示效果的差異大小 (e. g. $\mu_1 - \mu_2$)
 - 檢定水準 (α level, Prob. of Type I error)
 - 檢定力 ($1 - \beta$, Power of the test)
- 

$$2\sigma^2(z_\alpha + z_{1-\beta})^2 / (\mu_1 - \mu_2)^2 = 13.44$$

檢定兩組平均值的差異時 實驗動物樣本數的估算

$$n_1 = n_2 = 2\sigma^2(z_\alpha + z_{1-\beta})^2 / (\mu_1 - \mu_2)^2$$

- e.g. 某測量值的變異數為0.64，兩組平均值的差異在1.2以上才是有意義的，在雙尾檢定水準為.05且檢力為.90的條件下，每組需要多少隻動物？

$$n_1 = n_2 = 2(0.64)(1.96 + 1.28)^2 / (1.2)^2 = 9.33 \approx 10$$

● 若改為單尾檢定則


$$n_1 = n_2 = 2(0.64)(1.65 + 1.28)^2 / (1.2)^2 = 7.63 \approx 8$$

● 若變異數較大 $\sigma^2 = 1$ ，則

$$n_1 = n_2 = 2(1)(1.96 + 1.28)^2 / (1.2)^2 = 14.58 \approx 15$$


● 若兩組有意義的差異較小

$$n_1 = n_2 = 2(0.64)(1.96 + 1.28)^2 / (1)^2 = 13.44 \approx 14$$



例：某研究擬用大白鼠進行實驗，時驗共分**9**組，因考慮實驗過程中有些白鼠會因感染而死亡，故提出要使用**200**隻大白鼠

- 這樣的大白鼠數量要求合理嗎？
- 研究者應提供哪些資訊？
 - 若不考慮感染，原本需要多少隻？
 - 感染率為何？
 - 總共需要多少隻？



其他與控制實驗動物用量相關的方法

- 重複測量
- 連續性實驗及統計分析



結 論

● 「樣本數愈大愈好」？

No!

適當最好！