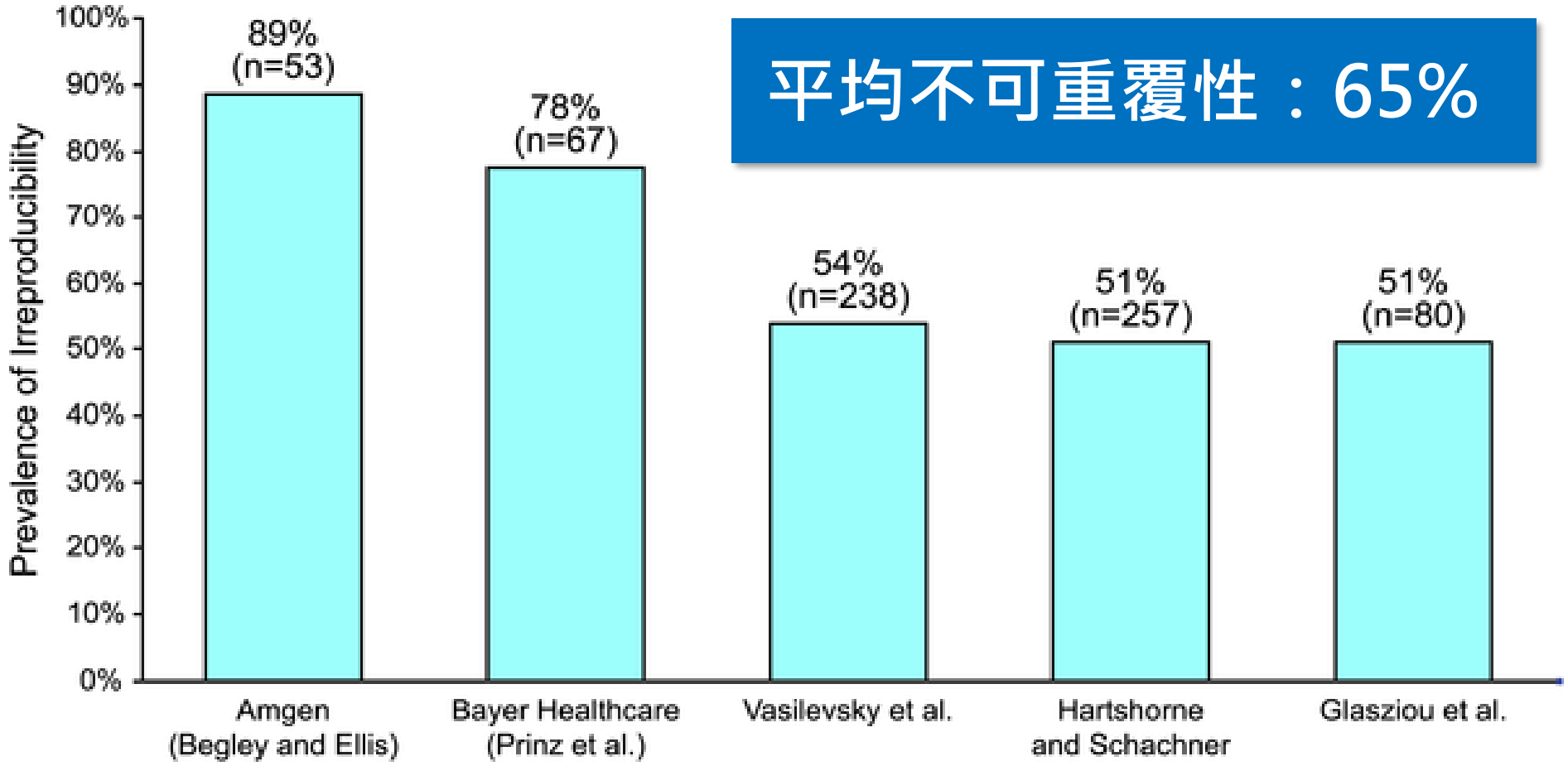
好的動物實驗規劃
由PREPARE及ARRIVE開始

國家實驗動物中心 秦咸靜主任

NARLabs
國家實驗研究院

動物實驗的挑戰- 再現性不足



平均不可重覆性：65%

Freedman LP, Cockburn IM, Simcoe TS (2015)
The Economics of Reproducibility in Preclinical Research.
PLoS Biol 13(6): e1002165. doi:10.1371/journal.pbio.1002165

PLOS BIOLOGY

動物實驗成果發表- 欠缺實驗細節

OPEN ACCESS Freely available online

PLoS one

Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals

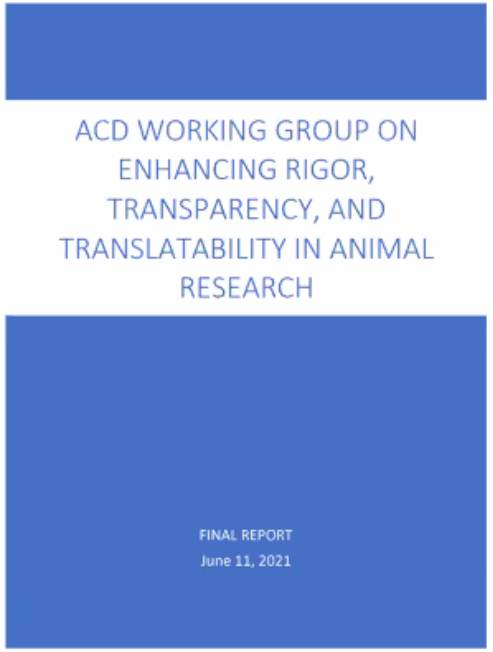
Carol Kilkenny[1]*, Nick Parsons[2], Ed Kadyszewski[3], Michael F. W. Festing[4], Innes C. Cuthill[5], Derek Fry[6], Jane Hutton[7], Douglas G. Altman[8]

- The survey identified key areas for improvement:

Experimental design	Statistical analysis	Reporting of studies
Most papers did not report randomisation (88%) or blinding (86%) to reduce bias in animal selection and outcome measurements.	Only 70% of publications fully described statistical methods and presented the result with a measure of variability.	Only 59% included three important pieces of information: hypothesis, number of animals and characteristics of animals.

NIH建議採用ARRIVE Essential 10

NIH Encourages the Use of the ARRIVE Essential 10 Checklist in all Publications Reporting on the Results of Vertebrate Animal and Cephalopod Research



NIH National Institutes of Health
Turning Discovery Into Health

When ARRIVE 2.0 should be followed:

Writing stage

AND

Entire research process

"Strengthening these elements **across the life of a study, from planning to execution and publication,** will result in a higher-quality knowledge base and will better inform future research."

實驗規劃

執行計畫

成果發表

6

ARRIVE

The ARRIVE guidelines are endorsed by journals, funders and learned societies.

Journals

PLOS

nature

BioMed Central
The Open Access Publisher

eLIFE

Over 400 journals have incorporated the ARRIVE guidelines in their Instructions to Authors

Funders

MRC Medical Research Council

wellcome trust

BBSRC
bioscience for the future

amrc
ASSOCIATION OF MEDICAL RESEARCH CHARITIES

The major funding bodies of biomedical research in the UK support the ARRIVE guidelines.

Universities

MANCHESTER 1824
The University of Manchester

UCL

St George's
University of London

UNIVERSITY OF BATH

Newcastle University

UNIVERSITY OF CAMBRIDGE

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

Universities endorse the ARRIVE guidelines by encouraging staff and students to use the guidelines.

Learned Societies

BPS BRITISH PHARMACOLOGICAL SOCIETY

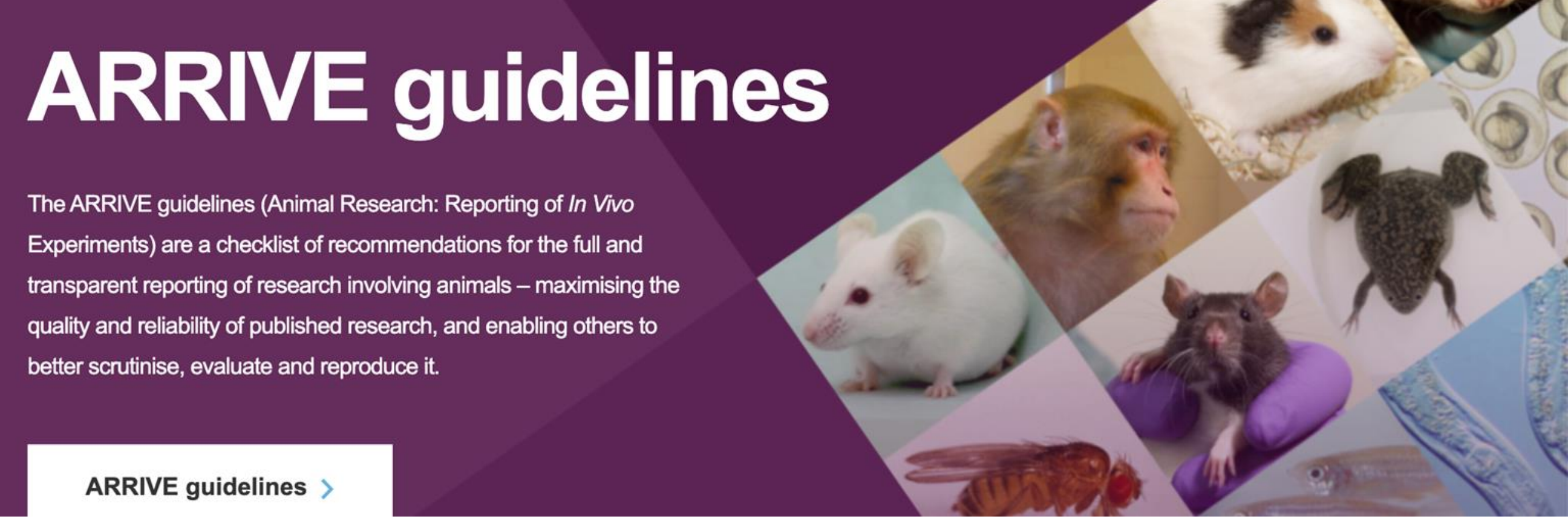
SEB
Society for Experimental Biology

SOCIETY OF Biology

British Society for immunology

The Physiological Society

A growing number of learned societies endorse the ARRIVE guidelines and share the guidelines with their members.



ARRIVE指南目標在改善動物實驗成果報導品質

不是注重吸引人的實驗成果，而是注重嚴謹的實驗規劃與執行細節，增加可信度

Title

1. Accurate & concise description

Abstract

2. Background, objectives, methods, key findings and conclusions

Introduction

3. Background

4. Objectives

Methods

5. Ethical statement

6. Study design (blinding/randomisation)

7. Experimental procedures (How? When? Where? Why?)

8. Experimental animals (species, sex, weight)

9. Housing and husbandry

10. Sample size

11. Allocation experimental groups

12. Experimental outcomes

13. Statistical methods

Results

14. Baseline Data

15. Numbers Analysed

16. Outcomes & estimation

17. Adverse events

Discussion

18. Interpretation & implications

19. Generalisability and translation

20. Funding

ARRIVE

ARRIVE GUIDELINE

- **ARRIVE 1.0 - 如何發表動物實驗相關之論文**
- **ARRIVE 2.0 (Essential 10)- 精簡為10項在計畫申請時應完備的規劃項目**
- **好的科學不光是要做到，還要讓別人看到**

1. 實驗設計	4. 逢機 Randomisation	7. 統計方法
2. 樣本數	5. 盲法 Blinding	8. 實驗動物
3. 樣本納入及排除	6. 結果評量指標	9. 實驗程序
		10. 結果

PREPARE GUIDELINE

PREPARE: guidelines for planning animal research and testing

Adrian J Smith[1], R Eddie Clutton[2], Elliot Lilley[3], Kristine E Aa Hansen[4] and Trond Brattelid[5]

Laboratory Animals
2018, Vol. 52(2) 135–141

DOI: 10.1177/002367721
journals.sagepub.com/h
SAGE

Abstract

There is widespread concern about the quality, reproducibility and translatability of studies involving r animals. Although there are a number of reporting guidelines available, there is very little overarchi ance on how to *plan* animal experiments, despite the fact that this is the logical place to start ensurin In this paper we present the PREPARE guidelines: Planning Research and Experimental Pro on Animals: Recommendations for Excellence. PREPARE covers the three broad areas which de the quality of the preparation for animal studies: formulation, dialogue between scientists and the facility, and quality control of the various components in the study. Some topics overlap and the P checklist should be adapted to suit specific needs, for example in field research. Advice on use of th list is available on the Norecopa website, with links to guidelines for animal research and testing, a norecopa.no/PREPARE.

The PREPARE guidelines cover 15 topics, grouped in three main sections:

A. Formulation of the study
1. Literature searches
2. Legal issues
3. Ethical issues, harm-benefit assessment and humane endpoints
4. Experimental design and statistical analysis

B. Dialogue between scientists and the animal facility
5. Objectives and timescale, funding and division of labour
6. Facility evaluation
7. Education and training
8. Health risks, waste disposal and decontamination

C. Quality control of the components in the study
9. Test substances and procedures
10. Experimental animals
11. Quarantine and health monitoring
12. Housing and husbandry
13. Experimental procedures
14. Humane killing, release, reuse or rehoming
15. Necropsy

PREPARE GUIDELINE

- PREPARE (**P**lanning **R**esearch and **E**xperimental **P**rocedures on **A**nimals: **R**ecommendations for **E**xcellence)

- **動物實驗開始前應檢視的15個重點項目**

1. 系統性文獻檢索	6. 動物設施評估	11. 檢疫及健康監測
2. 法規	7. 教育訓練	12. 飼養管理
3. 倫理- 3R之落實模式	8. 環安, 生安及職安	13. 實驗流程
4. 實驗設計及統計	9. 測試物質	14. 人道終點與再應用
5. 實驗專案管理	10. 實驗動物	15. 解剖及採樣

PREPARE

norecopa

The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith[a], R. Eddie Clutton[b], Elliot Lilley[a], Kristine E. Aa. Hansen[c] & Trond Brattelid[a]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; [d]Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; [e]Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

PREPARE[1] consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE[2].
PREPARE covers the three broad areas which determine the quality of the preparation for animal studies:

1. **Formulation of the study**
2. **Dialogue between scientists and the animal facility**
3. **Quality control of the components in the study**

The topics will not always be addressed in the order in which they are presented here, and some topics overlap. The PREPARE checklist can be adapted to meet special needs, such as field studies. PREPARE includes guidance on the management of animal facilities, since in-house experiments are dependent upon their quality. The full version of the guidelines is available on the Norecopa website, with links to global resources, at **https://norecopa.no/PREPARE**.
The PREPARE guidelines are a dynamic set which will evolve as more species- and situation-specific guidelines are produced, and as best practice within Laboratory Animal Science progresses.

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. ☐ Assess the reproducibility and translatability of the project.
2. Legal issues	☐ Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. ☐ Locate relevant guidance documents (e.g. EU guidance on project evaluation).
3. Ethical issues, Harm-Benefit Assessment and humane endpoints	☐ Construct a lay summary. ☐ In dialogue with ethics committees, consider whether statements about this type of research have already been produced. ☐ Address the 3Rs (Replacement, Reduction, Refinement) and the 3Ss (Good Science, Good Sense, Good Sensibilities). ☐ Consider pre-registration and the publication of negative results. ☐ Perform a Harm-Benefit Assessment and justify any likely animal harm. ☐ Discuss the learning objectives, if the animal use is for educational or training purposes. ☐ Allocate a severity classification to the project. ☐ Define objective, easily measurable and unequivocal humane endpoints. ☐ Discuss the justification, if any, for death as an end-point.
4. Experimental design and statistical analysis	☐ Consider pilot studies, statistical power and significance levels. ☐ Define the experimental unit and decide upon animal numbers. ☐ Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.

ARRIVE

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

[1]The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK, [2]School of Veterinary Science, University of Bristol, Bristol, UK, [3]School of Biological Sciences, University of Bristol, Bristol, UK, [4]National Heart and Lung Institute, Imperial College London, UK, [5]Centre for Statistics in Medicine, University of Oxford, Oxford, UK.

	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	

The ARRIVE guidelines. Originally published in *PLoS Biology*, June 2010[2]

動物實驗規劃流程

- 文獻回顧
- 替代方法
- 系統性文獻回顧

- 人員教育訓練
- 實驗動物選擇及來源
- 動物設施的運作
- 職安、環安及生安
- 3R之落實策略

動物實驗是不是**最後一個選項**？

實驗設計與流程**樣本及統計**

動物來源、飼養**動物實驗管理**

實驗執行及報導**量測指標及試驗終點**

- 實驗設計與分組
- 測試物質
- 樣本的選擇與排除
- 避免實驗偏誤 (盲法及逢機)
- 統計方法的選擇

- 動物實驗專案管理
- 量測指標的選擇
- 解剖與採樣
- 人道試驗終點
- 實驗數據的呈現

實驗規劃- STEP1 確認實驗主軸

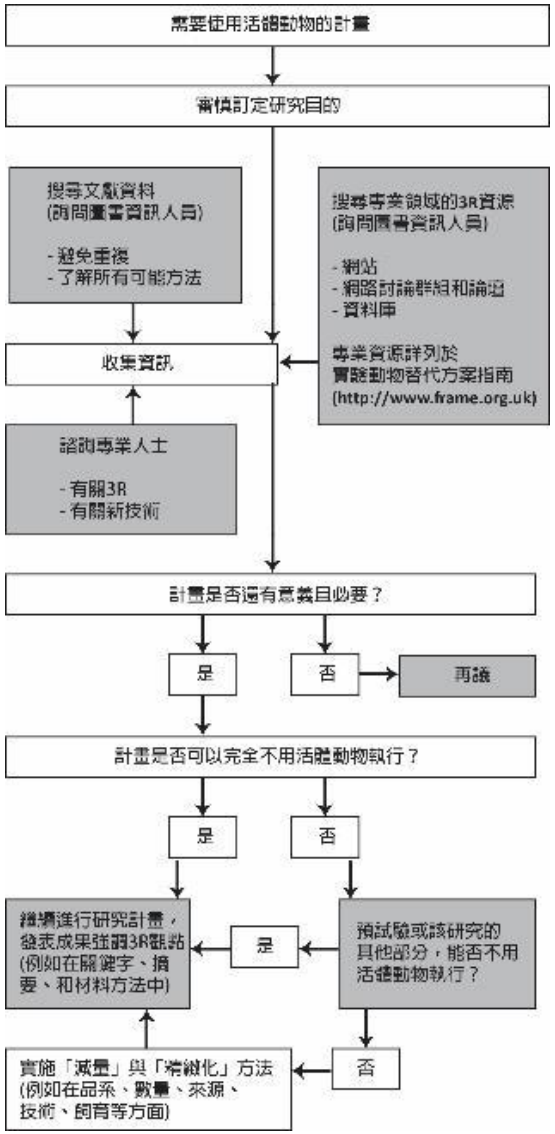
實驗標的

建立假說

動物模式

研究團隊

- 動物實驗規劃
  - 一定要使用動物嗎?
  - 動物模式能回答問題嗎?
  - **選擇正確的物種/ 品系**
  - 動物如何取得/ 運輸
  - **動物如何適應及飼養**
  - **良好的實驗設計**
  - **適當的統計方法**
  - **結果的呈現方式**



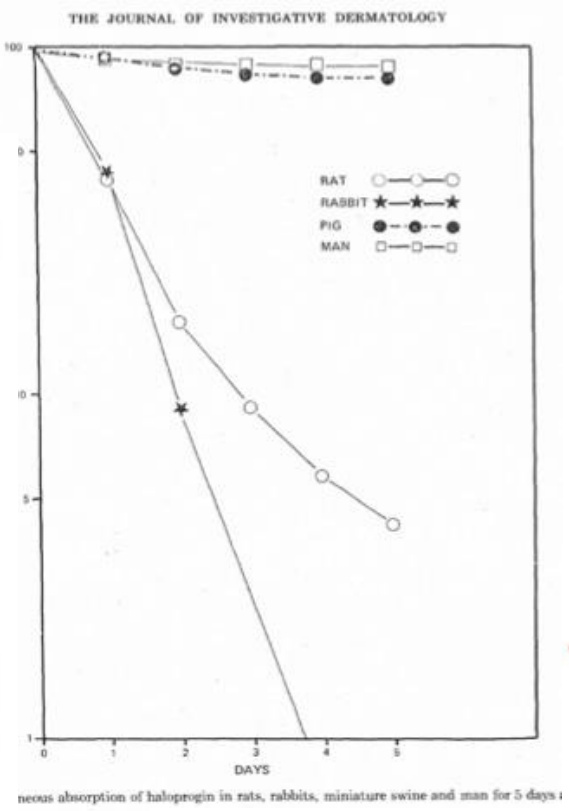
動物實驗是不是**最後一個選項**？

有系統地進行文獻回顧

- ✓文獻資料回顧目標
  - ✓ 明確的實驗目的
  - ✓ 資料庫：MEDLINE, TOXLINE, PUBMED, NCBI, AGRICOLA等
  - ✓ 實驗目的的檢索應包括替代方案的考量、及正反面實驗結果
  - ✓ 解釋所選用的動物模式如何能夠滿足研究目的
- ✓ 系統性文獻回顧 (Systematic Review) 與統合分析 (Meta-analysis) 可以更深入了解過往案例及差異

WHO IS IN THE STUDY?

- **實驗動物：**
  - 物種、品系、次品系、年齡、性別、體重、繁殖狀態、健康狀態
  - 來源 (供應商/ 品系碼/ 供應地)
  - 所用實驗動物是否
    - 是否適合進行實驗所需之操作
    - 是否具代表性



neous absorption of haloprogin in rats, rabbits, miniature swine and man for 5 days

(Bartek, LaBudde, Maibach, 1972)

Outbred 逢機品系 vs Inbred 近親品系

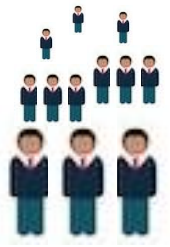
紐西蘭白兔

倉鼠

SD大鼠

ICR小鼠

outbred



Inbred 1

Inbred 2

B6小鼠

BALB/c小鼠

db/db小鼠

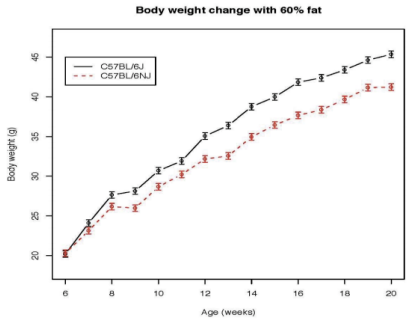
DBA小鼠

次品系: C57BL/6J vs C57BL/6N

C57BL/6**JNarl**	C57BL/6**NBltw**
NLAC NARLabs 國家實驗研究院 國家實驗動物中心 National Laboratory Animal Center	LASC 樂斯科生物科技

Metabolic Differences (DIO)

B6J gains more weight than B6NJ on high fat diet (HFD)



- C57BL/6J (000664) vs C57BL/6NJ (005304)
- Mice fed a 60 kcal% high fat diet
  - Beginning at 6 weeks of age

Nicholson, A et al. 2010. *Obesity* 18(10): 1902-1905. PMID: 20057372

Neurological Differences

Vision - Avoid Common Research Mistakes

C57BL/6N (*Crb1*^{rd8}); consequences of retinal degeneration

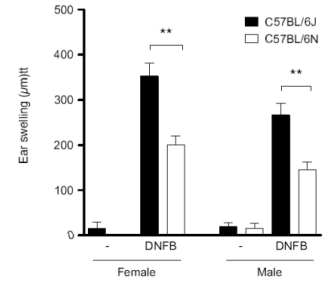
- Complication in interpretation of genes influencing diseases, phenotypes & developmental biology of sight & neurobiology
- Phenotypic analysis of genes implicated in cognitive function (behavioral tests that require visual cues)
- Research areas impacted:
  - Alzheimer's
  - Autism
  - Down Syndrome
  - Rhett Syndrome
  - Neurodegenerative disorders

Immunological Differences

B6J mice show greater DTH Response

Delayed Type Hypersensitivity (DTH) Response

- Sensitization and challenge with dinitrofluorobenzene (DNFB)
- B6J males & females show greater inflammatory response



Genetic Analysis

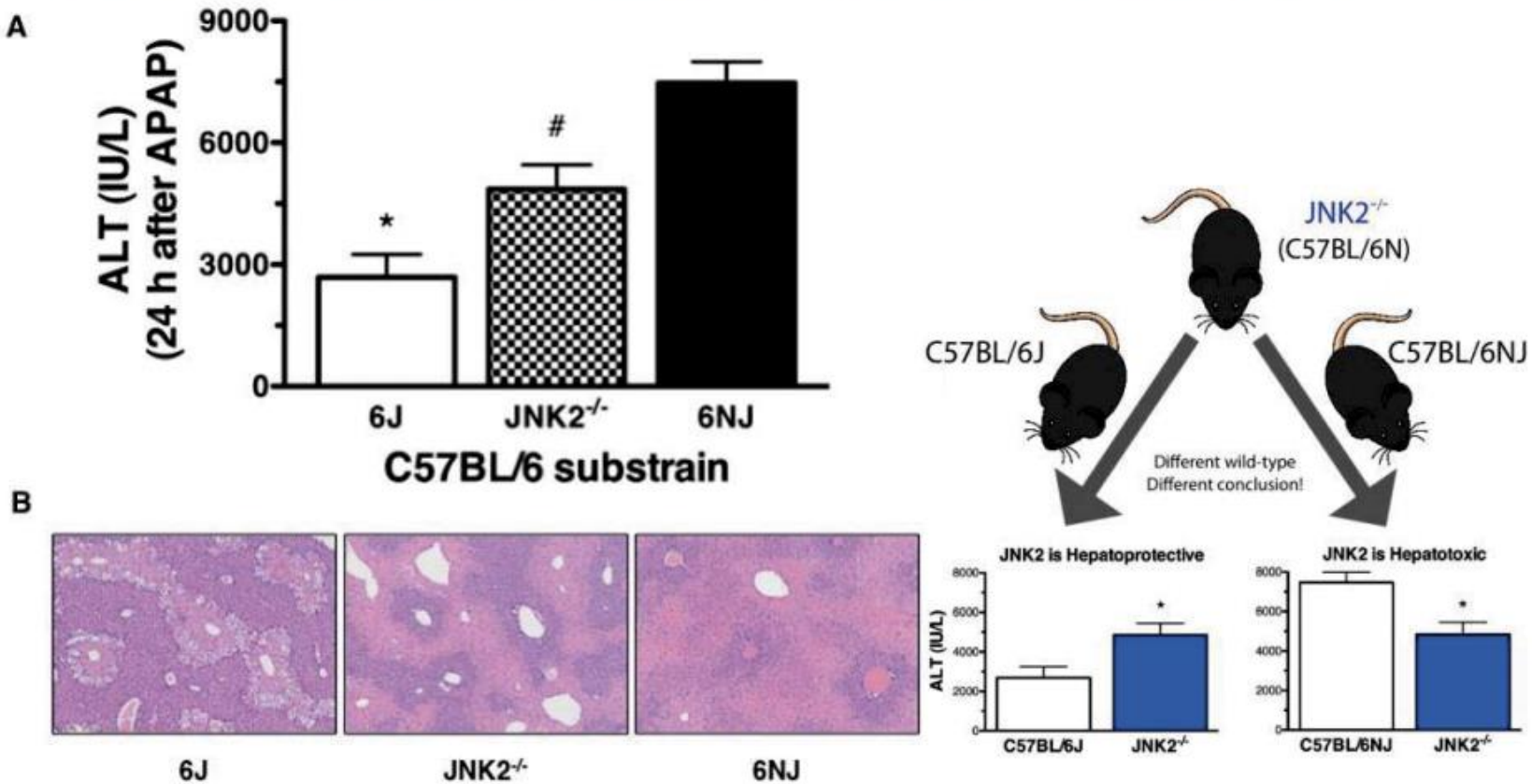
- Identified multiple SNPs & Indels
- Genomic structural variants

Simon, M. M., et al. (2013). *Genome Biology* 14(7): R82. PMID: 23902802

Select The Proper C57BL/6 Control

Avoid Common Research Mistakes

Effects of *Mapk9* (*Jnk2*) on acetominophen-induced liver injury (AILI)



Bourdi M et al. 2011. *Chem Res Toxicol* 24: 794-6. PMID:21557537

疾病模式與合作夥伴

自發性突變

肥胖及糖尿病 (db/db)

高血壓鼠 (SHR rat)

誘發式疾病模式

Covid-19 倉鼠模式

腦血管栓塞中風模式

脂肪肝及肝硬化模式

腫瘤移植模式

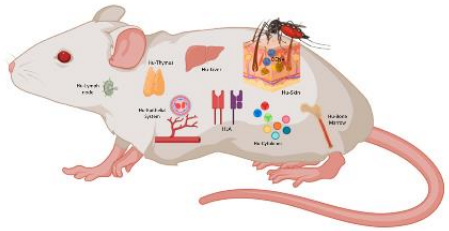
基因改造模式

自閉症/妥瑞症

多囊腎

罕見疾病

基因與疾病解碼



擬人化模式

源自病患腫瘤模式

人類免疫鼠

人類腸道菌鼠

在計畫規劃階段應該確認誰是你的神隊友? 訓練學生? 委託專家? 合作研究?

實驗規劃- STEP2 實驗樣本與統計方法

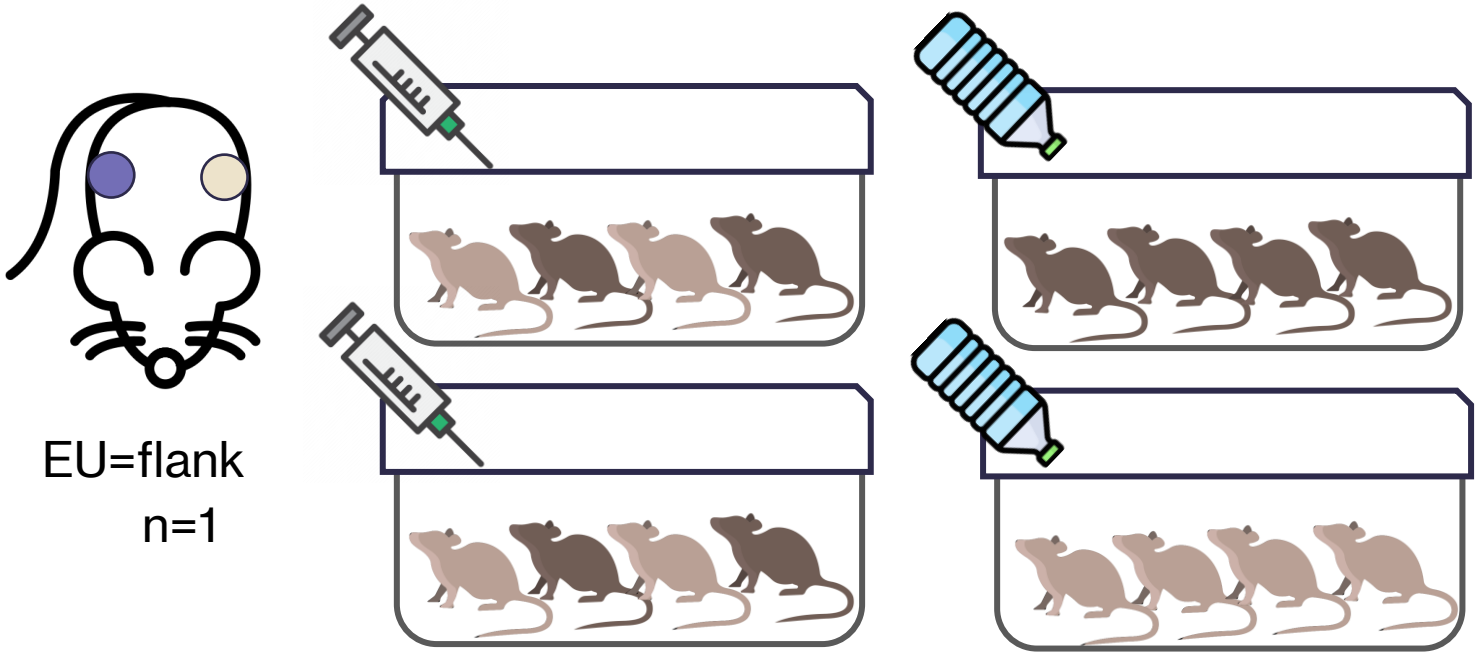
實驗單位

實驗與對照組

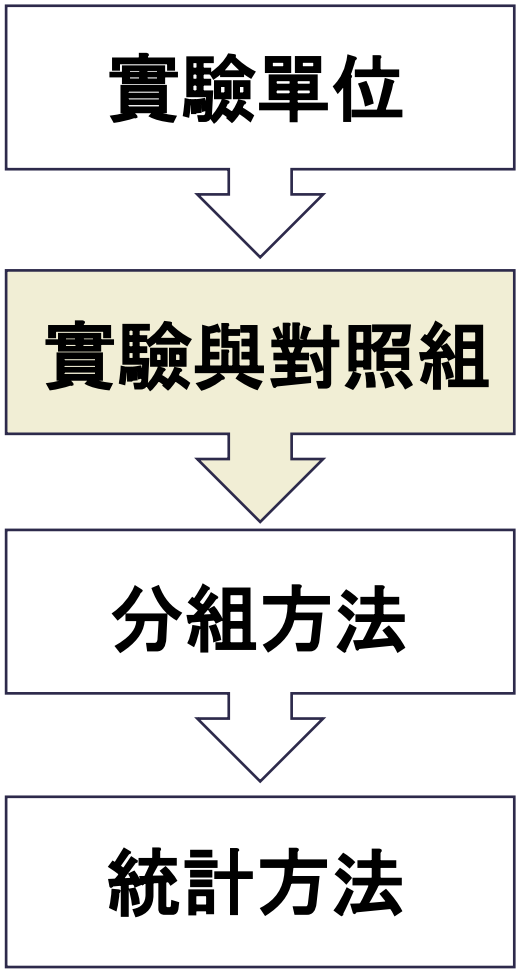
分組方法

統計方法

- 實驗單位 Experimental Unit (EU)
  - 統計分析的最小單位 (動物 / 籠 / 組)



實驗規劃- STEP2 實驗樣本與統計方法



- **對照組：**

提供一個可靠的參考點，排除已知或可能存在的未知變數影響

- 陽性對照組 (positive control):
  - 已知有變化 / 可做為比對標準，用來確保實驗正常運作
- 陰性對照組 (negative control):
  - 已知無變化 / 可做為比對標準，用來確保未知變數不影響實驗
- 空白對照組 (Mock) (Sham Control)
  - 模擬實驗組的過程，但質質未給予有效物質
- 媒介物對照組 (Vehicle control)
  - 實驗使用特殊溶劑時，通常會做媒介物對照

實驗規劃- STEP2 實驗樣本與統計方法

實驗單位

實驗與對照組

分組方法

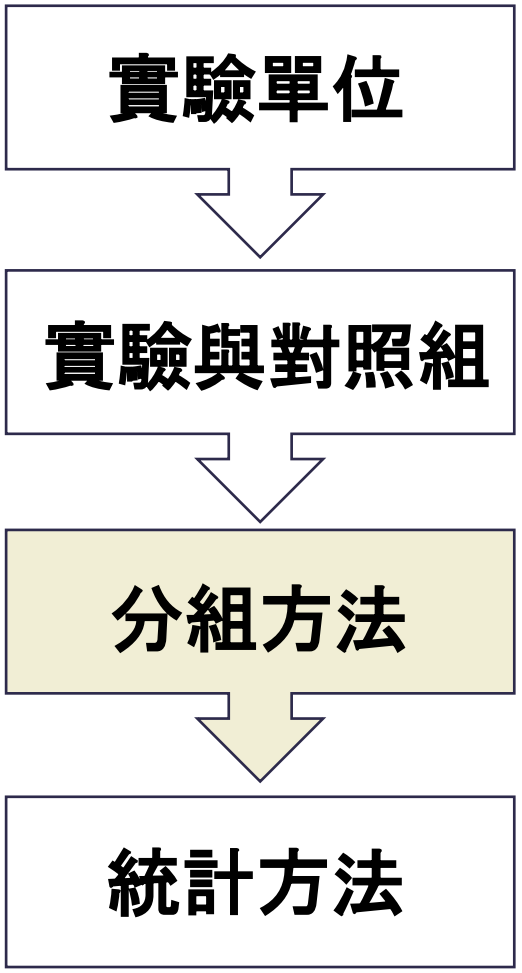
統計方法

逢機分組與盲法

-減少系統性偏誤，增加實驗可信度

- **Randomization 逢機分組**
  - 說明配置實驗單元 (EU) 到實驗組與對照組的做法
  - 說明減少潛在或人為偏誤的影響
- **Blinding 盲法**
  - 分組、操作、量測、資料分析，任一階段
  - 尤其是病理分析、行為分析等需操作者判斷時
  - 說明如何避免個人判斷造成的偏誤

實驗規劃- STEP2 實驗樣本與統計方法

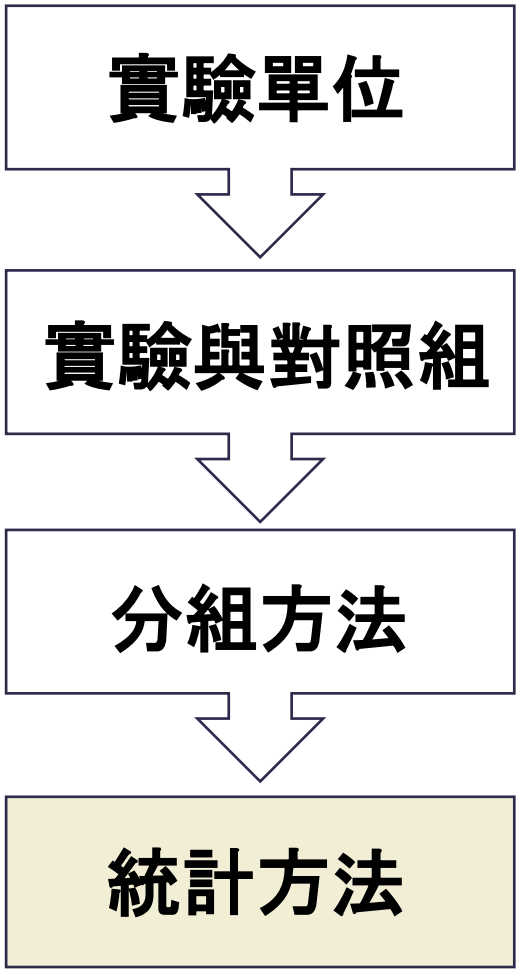


樣本的納入與排除

-預先決定最佳樣本範圍，避免依想要結果挑選樣本

- **Inclusion 樣本納入的原則**
  - 定義主要的目標族群及其具備的重要指標或特質
  - 例如：腫瘤體積超過 100mm^3
- **Exclusion 樣本排除的原則**
  - 定義會干擾實驗結果的狀況
  - 例如：動物瀕死、設備問題

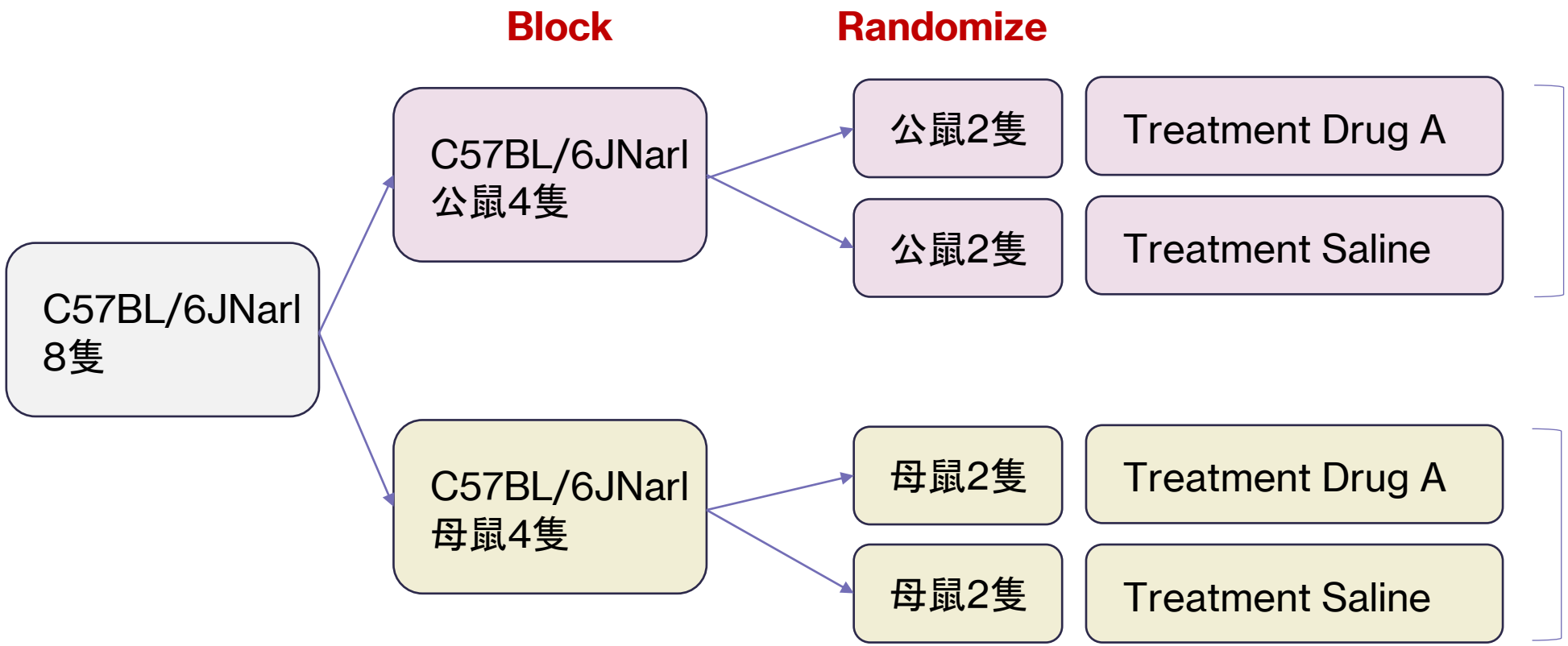
實驗規劃- STEP2 實驗樣本與統計方法



- 前導試驗- 依過往經驗，探索實驗方法及數據態樣
  - Pilot Study 預試驗
  - Exploratory Study 探索型研究
- 正式試驗- 驗證假說，需要達到顯著差異
  - Confirmatory Study
  - 運用前導試驗結果來進行樣本數預估
- 重要考量因子
  - Type of comparison
  - Primary variables: continuous/ categorical
  - Errors: type I/ type II

Randomized block design

目的：預先依照特定變因分類，減少已知變因對於實驗結果的影響



EU= mice
N=2

比較 treatment
一項變因

比較 treatment
一項變因

Factorial Experimental Design

- 快速篩選多個因子對實驗的影響 (2^k, k=factors)
- 可協助判斷那一個因子影響結果、判斷最適量、決定 sample size
- 一個資料多用途，資料最大化，減少動物使用 (share N)

	Control	Drug
Male	 *a*	 *c*
Female	 *b*	 *d*

EU= mice, N=2

比較- treatment及性別二項變因 (2^2)

因此

1. **共有4個組別，有4個mean (*a, b, c, d*)**
2. **Treatment差異：(*c+d*)/2- (*a+b*)/2**
3. **性別差異 : (*a+c*)/2- (*b+d*)/2**
4. **性別對Treatment的影響 ((a-c)-(b-d))/2**

quantitative variable vs categorical variable

multi-way ANOVA vs ?

Experimental Design Assistant

National Centre for the Replacement Refinement & Reduction of Animals in Research

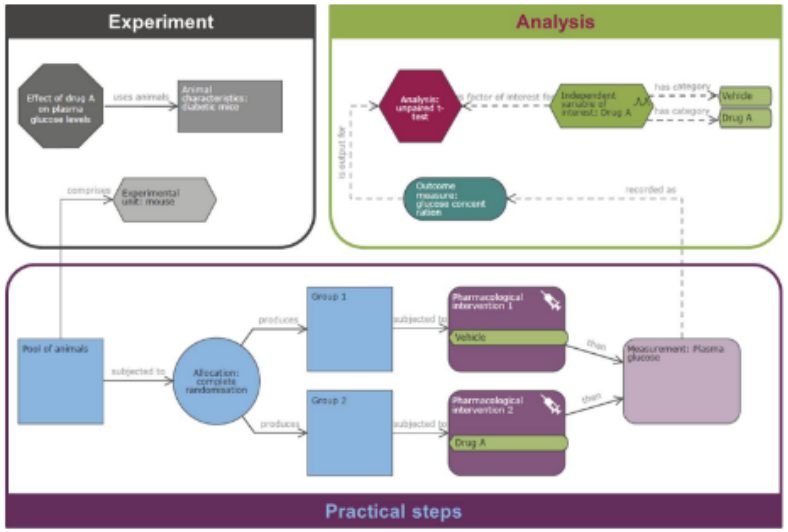
HOME ABOUT EDA USER GUIDE EXPERIMENTAL DESIGN FEEDBACK EDA APP SEARCH

Welcome to the Experimental Design Assistant, a free resource from the NC3Rs to support researchers in the planning of animal experiments - ensuring robust study design and reliable and reproducible findings.

The EDA consists of a web application and a supporting website; benefits include:

- The ability to build a stepwise visual representation of your experiment
- Feedback and advice on your experimental plan
- Dedicated support for randomisation, blinding and sample size calculation
- Practical information to improve knowledge of experimental design
- Improved transparency of your experimental design, allowing you to share and discuss your plan with colleagues and collaborators

Check the **video tutorials** and the **user guide** for general information on the EDA process. Find out more about the background for this project.



Step 1

Login or Register

Start using the EDA application

Step 2

Plan your experiment as a diagram

Check the examples and the user guide for more information

Step 3

Critique your design

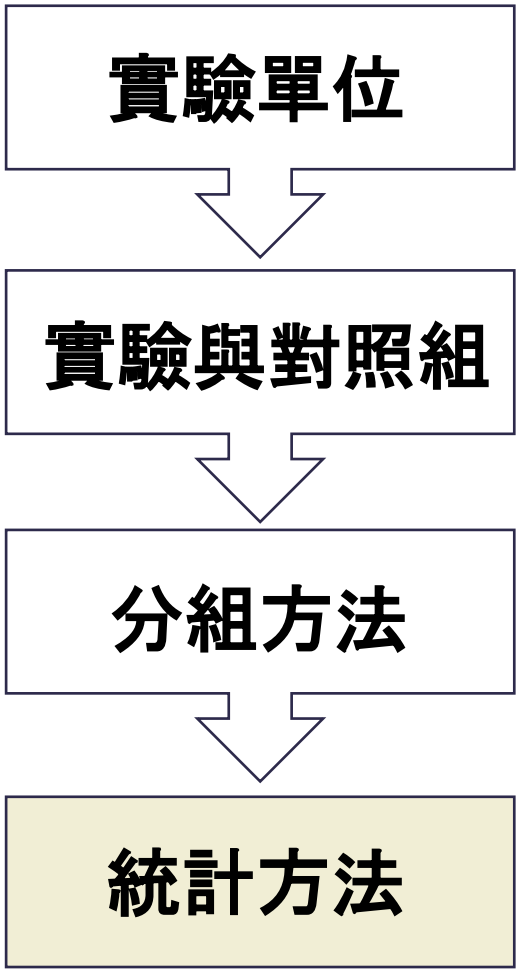
The critique function enables you to get feedback and advice on your diagram, find more information here

Step 4

Improve your design

Modify your experimental plan based on feedback from the system

實驗設計- STEP2 實驗樣本與統計方法

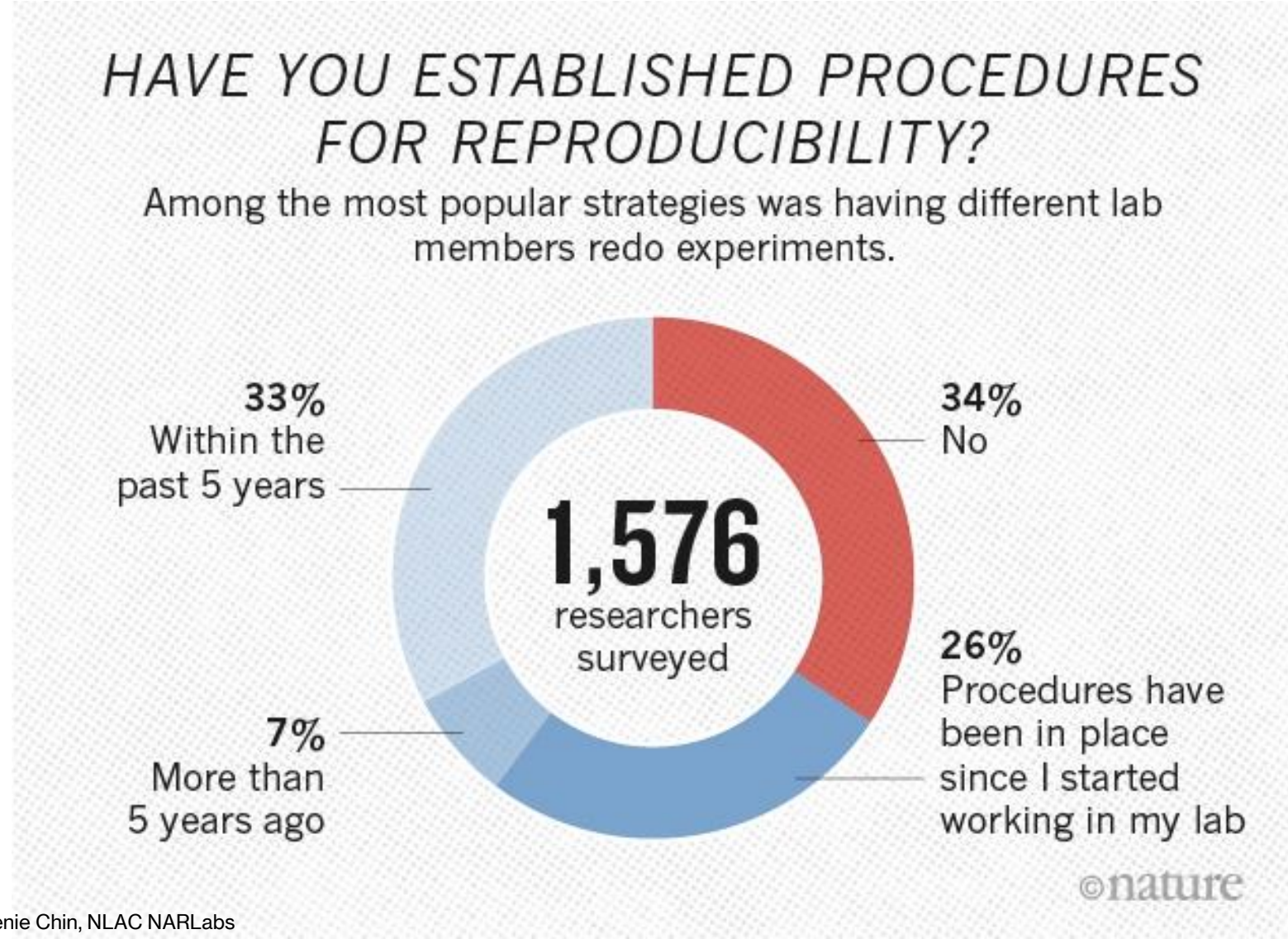


樣本數計算

- 樣本數 (N) 在整個實驗期間及報告撰寫時，都應該一致 (若要排除應符合排除原則，並說明)
- 運用前導試驗結果來進行樣本數預估 (用那一項指標為基準做樣本數預估?)
- 減量原則：在可得最大科學價值下，採用最少傷害的方法與最少的動物隻數

  (數量太多浪費多餘的動物生命，數量太少浪費全部的動物生命)
- 不適合的做法
  - 採用文獻上未經驗證的樣本數
  - 不解釋或硬套公式

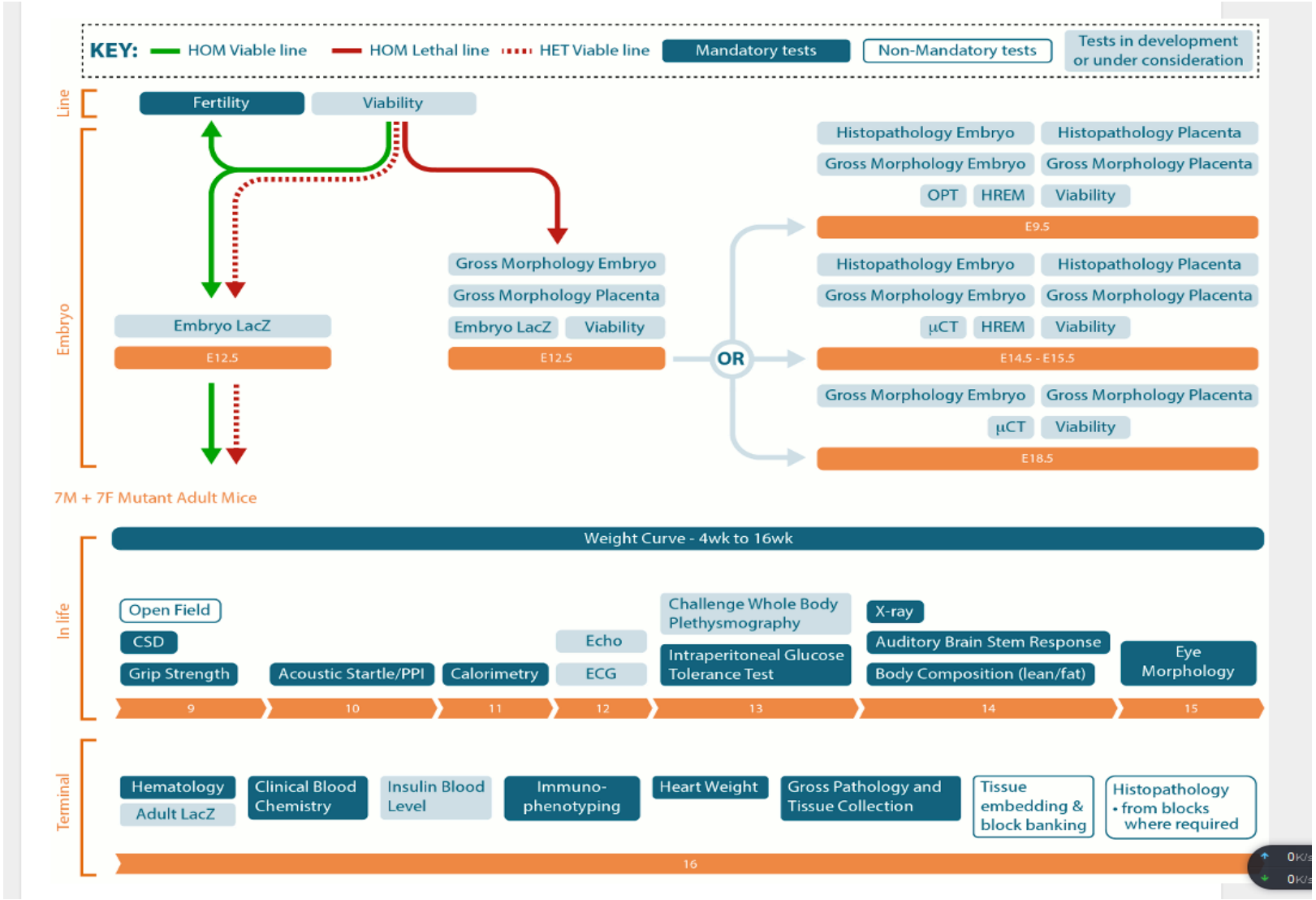
實驗規劃- STEP3 實驗PROTOCOL



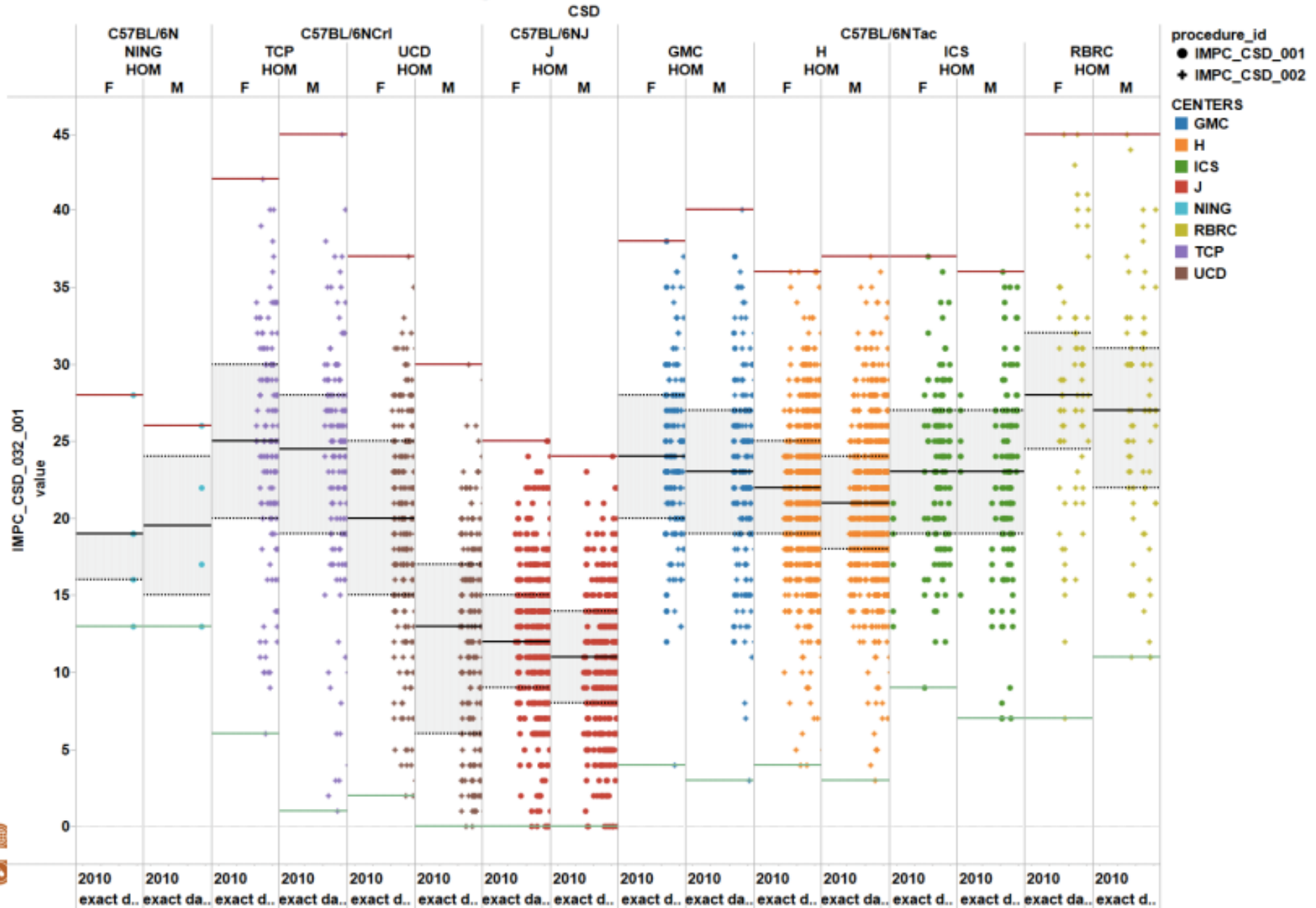
IMPC

International Mouse Phenotyping Consortium

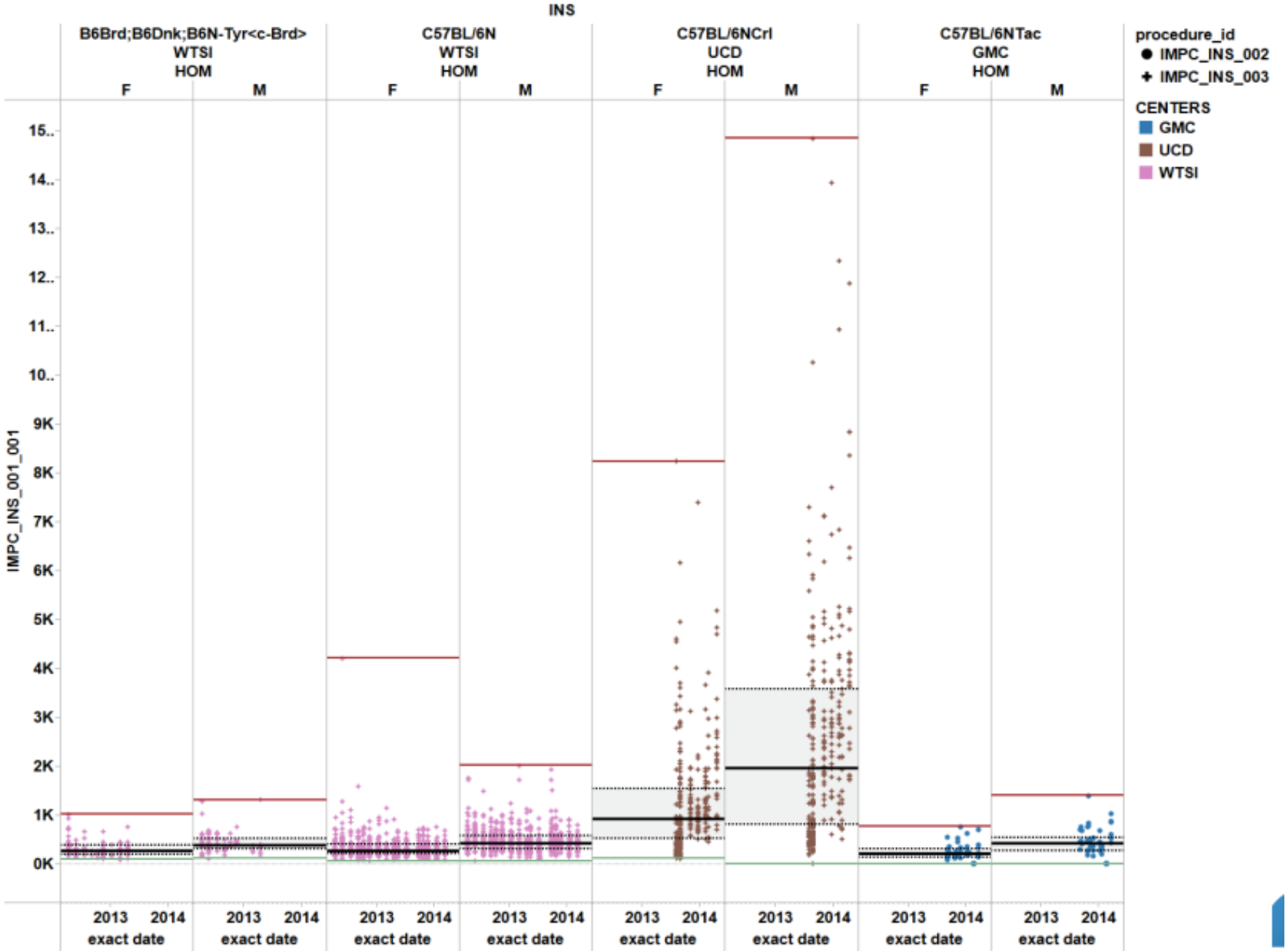
The Adult and Embryonic Phenotype Pipeline



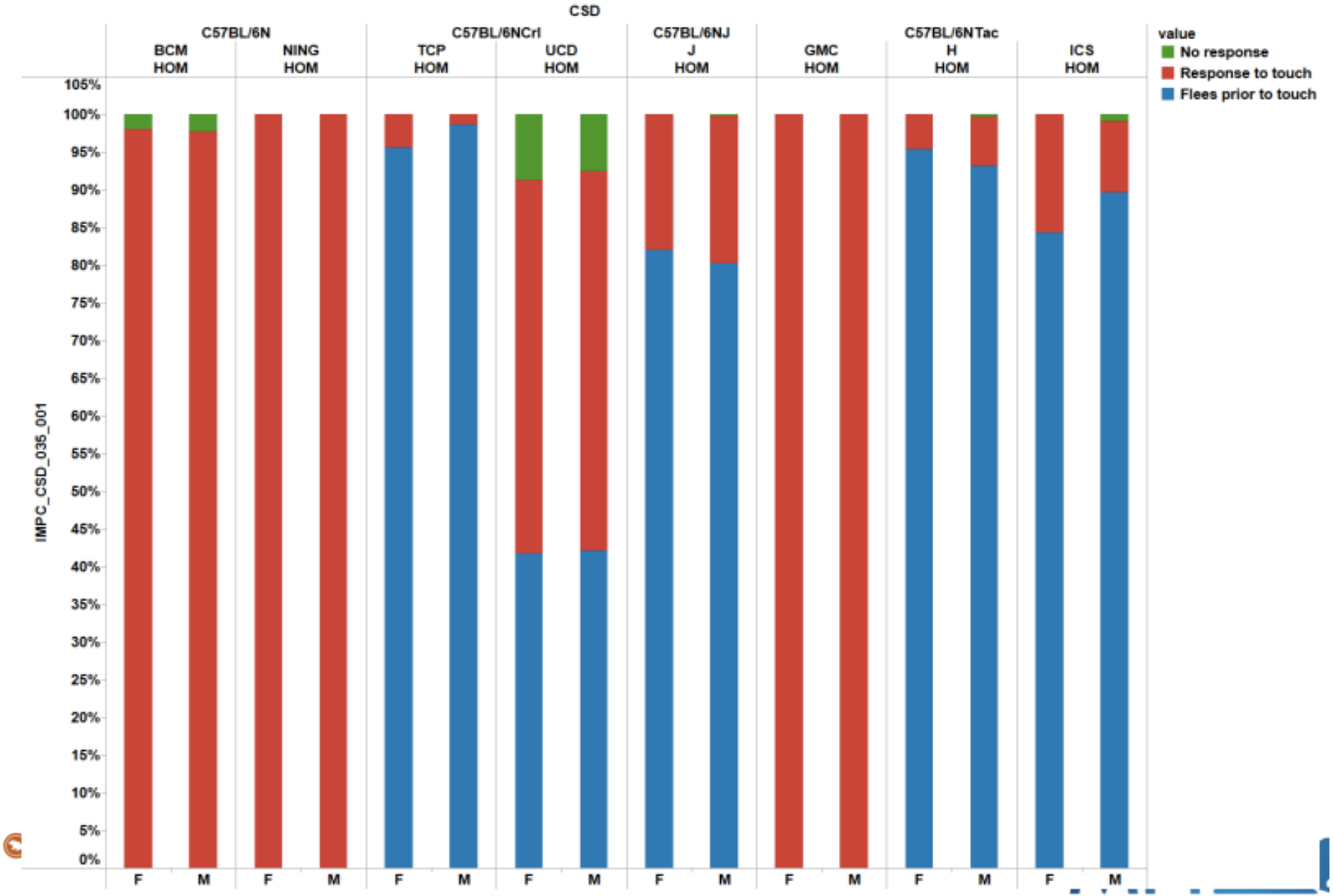
Locomotor activity IMPC_CSD_032_001



Insulin IMPC_INS_001_001



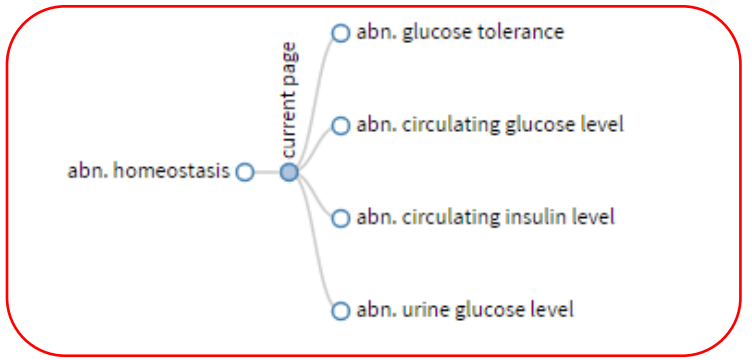
Touch escape IMPC_CSD_035_001



Phenotype: abnormal glucose homeostasis

Definition	anomaly in the processes involved in the maintenance of an internal equilibrium of glucose in the fluids and tissues
Synonyms	abnormal glucose metabolism, metabolism: abnormal glucose homeostasis
Computationally mapped HP term	• Abnormal glucose homeostasis
Procedure	• Simplified IPGTT (ESLIM, v1)
MGI MP browser	MP:0002078

Login to register interest



Phenotype associations stats

9.62% of tested genes with null mutations on a B6N genetic Select a parameter

EMPReSS	Title: Simplified Intra-Peritoneal Glucose Tolerance Test (I.P.G.T.T)	
Standard Operating Procedure	Doc. Number: ESLIM_004_001 Rev No. 1	Date Issued: 01/06/04

1.0 Purpose:

1.1 The glucose tolerance test measures the clearance of an intraperitoneally injected glucose load from the body. Animals are fasted for approximately 16 hours, a solution of glucose is administered by intraperitoneal (IP) injection and blood glucose is measured at different time points during the following 2 hours.

2.0 Scope:

2.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure.

2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the Clinical Chemistry, Haematology and Metabolism department leader.

2.3 Any deviances from this protocol must be reported to the Clinical Chemistry, Haematology and Metabolism department leader.

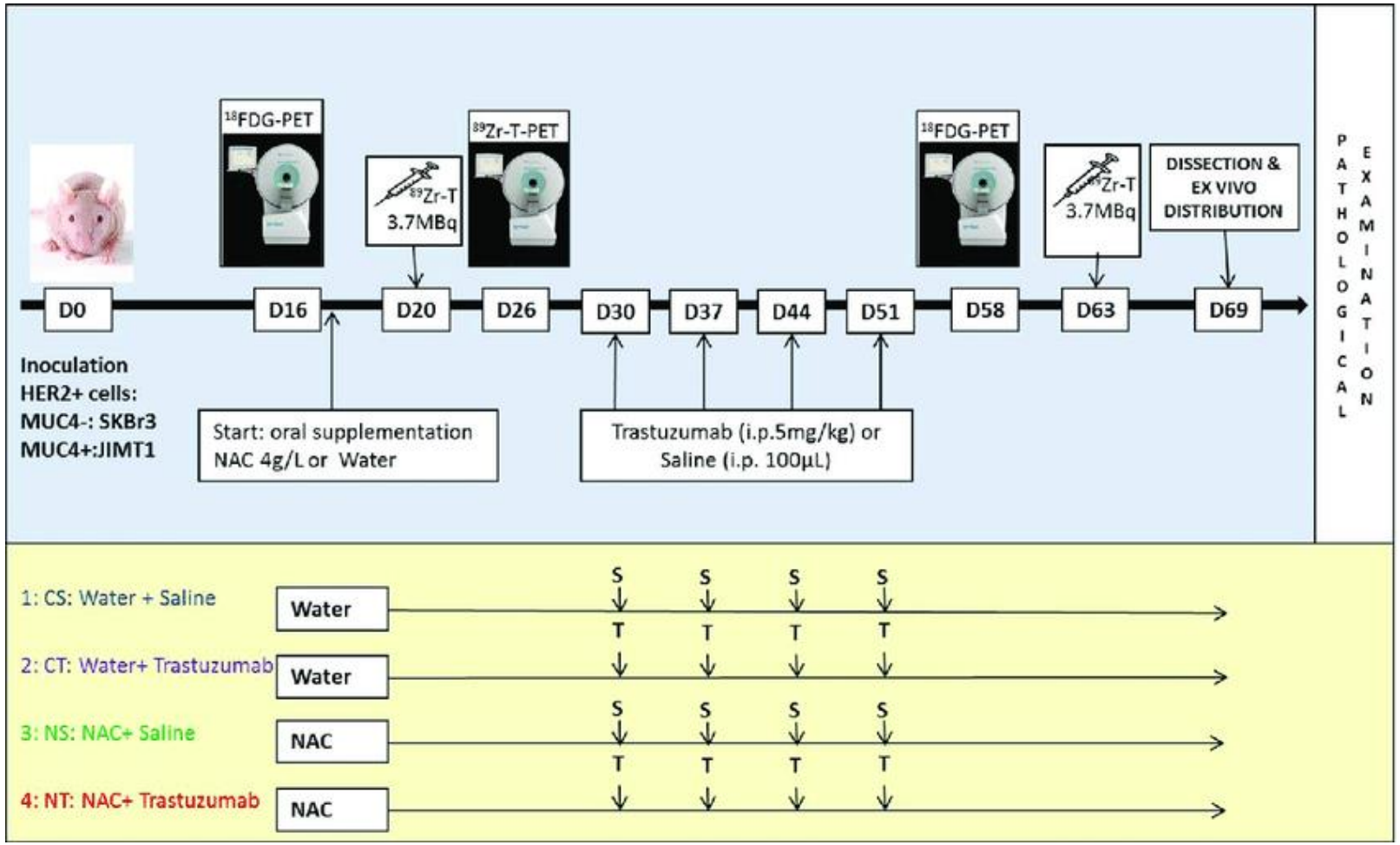
3.0 Safety Requirements:

3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

3.2 Dispose of blades, and needles in sharps waste.

4.0 Associated Documents:

實驗規劃- STEP4 實驗流程



What 實驗項目

How 實驗protocol

When/ how often 操作時序及頻率

Where 動物移動及適應期

Why 量測項目的選擇

動物實驗的良莠:
直接影響- 實驗操作
間接影響- 動物飼養管理

實驗規劃- STEP 5 動物實驗管理

繁殖育種
環境豐富化
健康品質
社群行為

動物資源
供應單位

動物運輸

動物實驗
執行單位

檢疫及適應
環境豐富化？
健康品質？
社群行為？

動物實驗

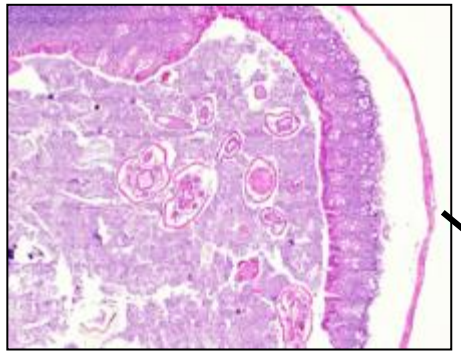
實驗操作
採樣分析

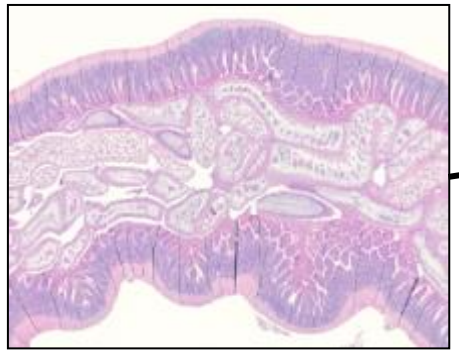
保定技術？
投藥方式？
採樣位置？
實驗及病理分析？

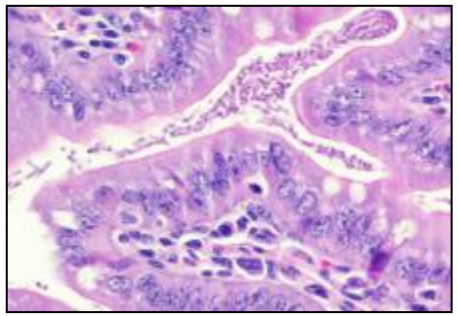
實驗設計

正確動物？
飼料及營養？
分組及統計？
飼育環境？

動物例行健康監測及檢疫

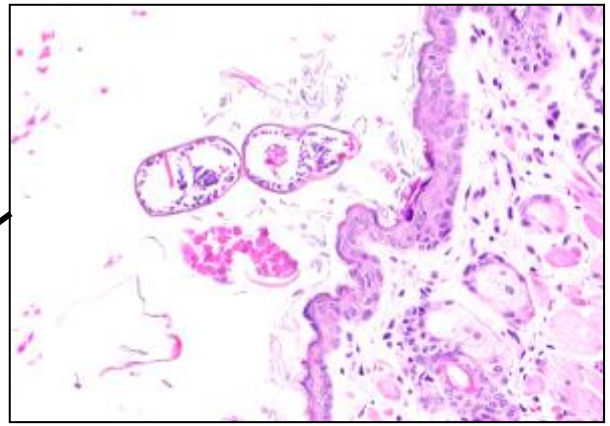




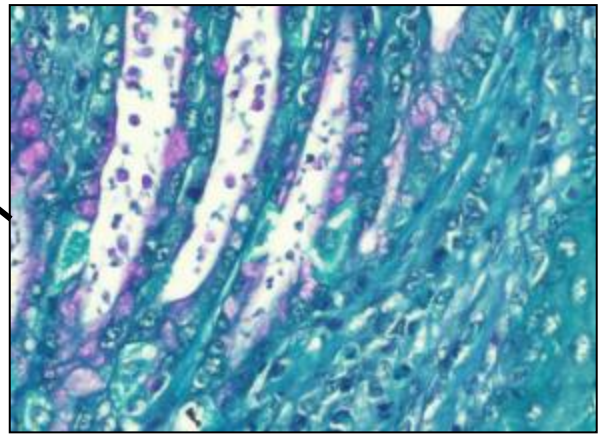


Cecum:
Pinworms

Small intestine:
Spironucleus spp.
Tapeworms

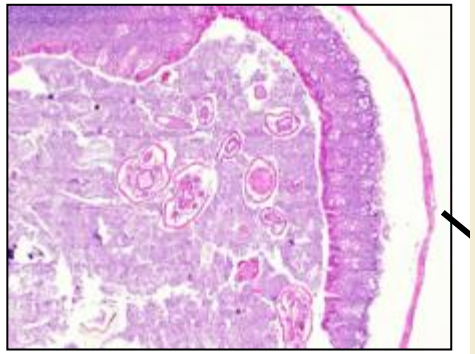


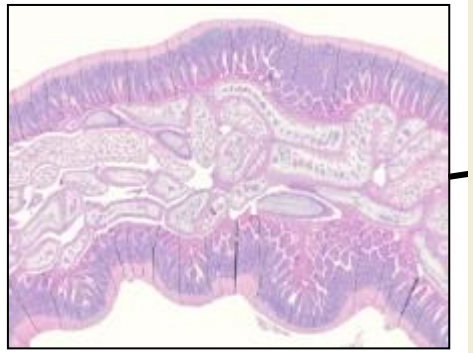
Skin: mite

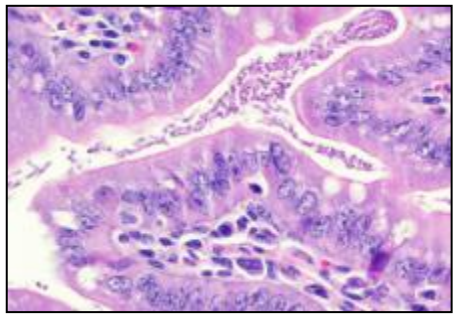


Stomach: Cryptosporidiosis

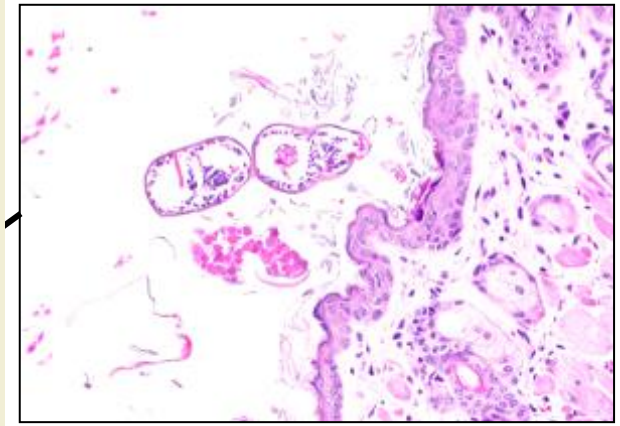
動物例行健康監測及檢疫



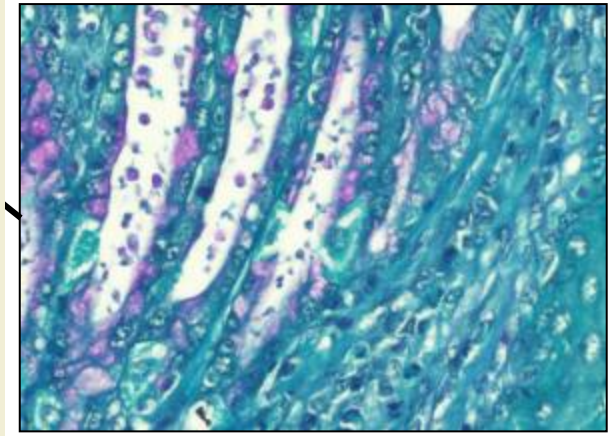




- Norovirus
- Helicobacter
- Mycoplasma
- MHV
- Pinworm



Skin: mite



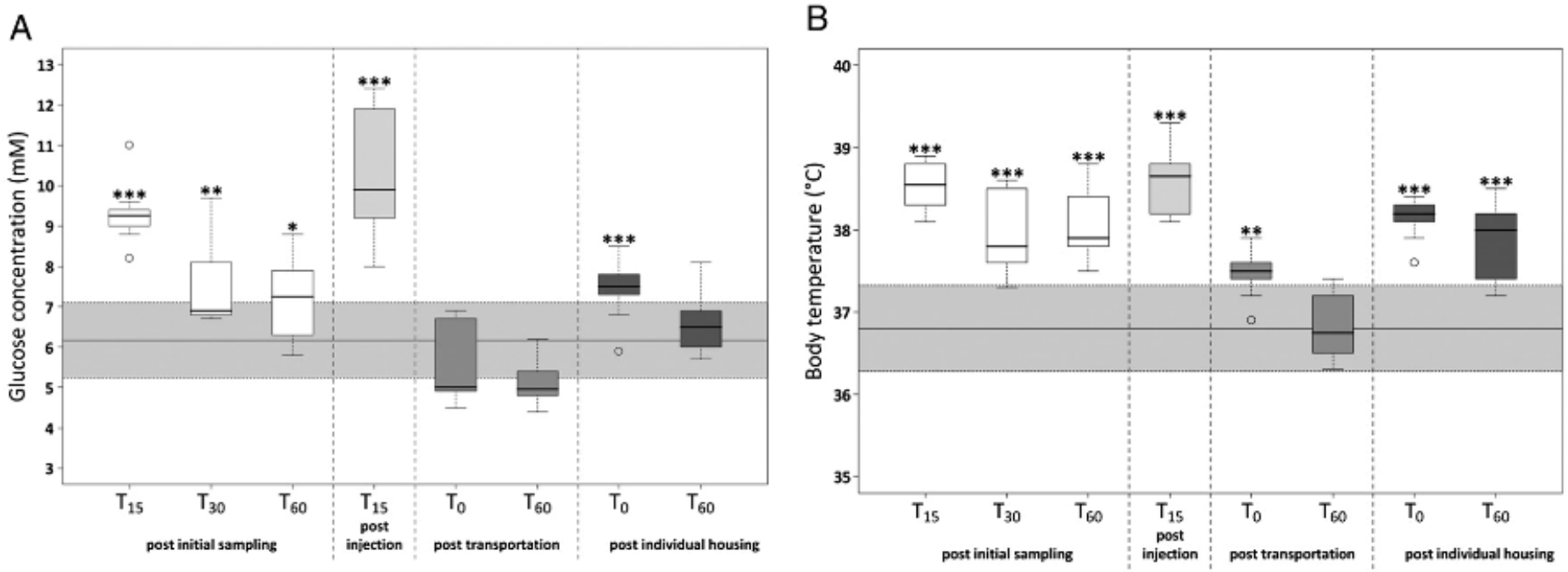
Stomach: Cryptosporidiosis

動物適應, 換籠及操作

Experimental and husbandry procedures as potential modifiers of the results of phenotyping tests

Anna-Karin Gerdin, Natalia Igosheva, Laura-Anne Roberson, Ozama Ismail, Natasha Karp, Mark Sanderson, Emma Cambridge, Carl Shannon, David Sunter, Ramiro Ramirez-Solis, James Bussell, Jacqueline K. White *

Mouse Genetics Project, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK



實驗規劃- STEP6 實驗結果

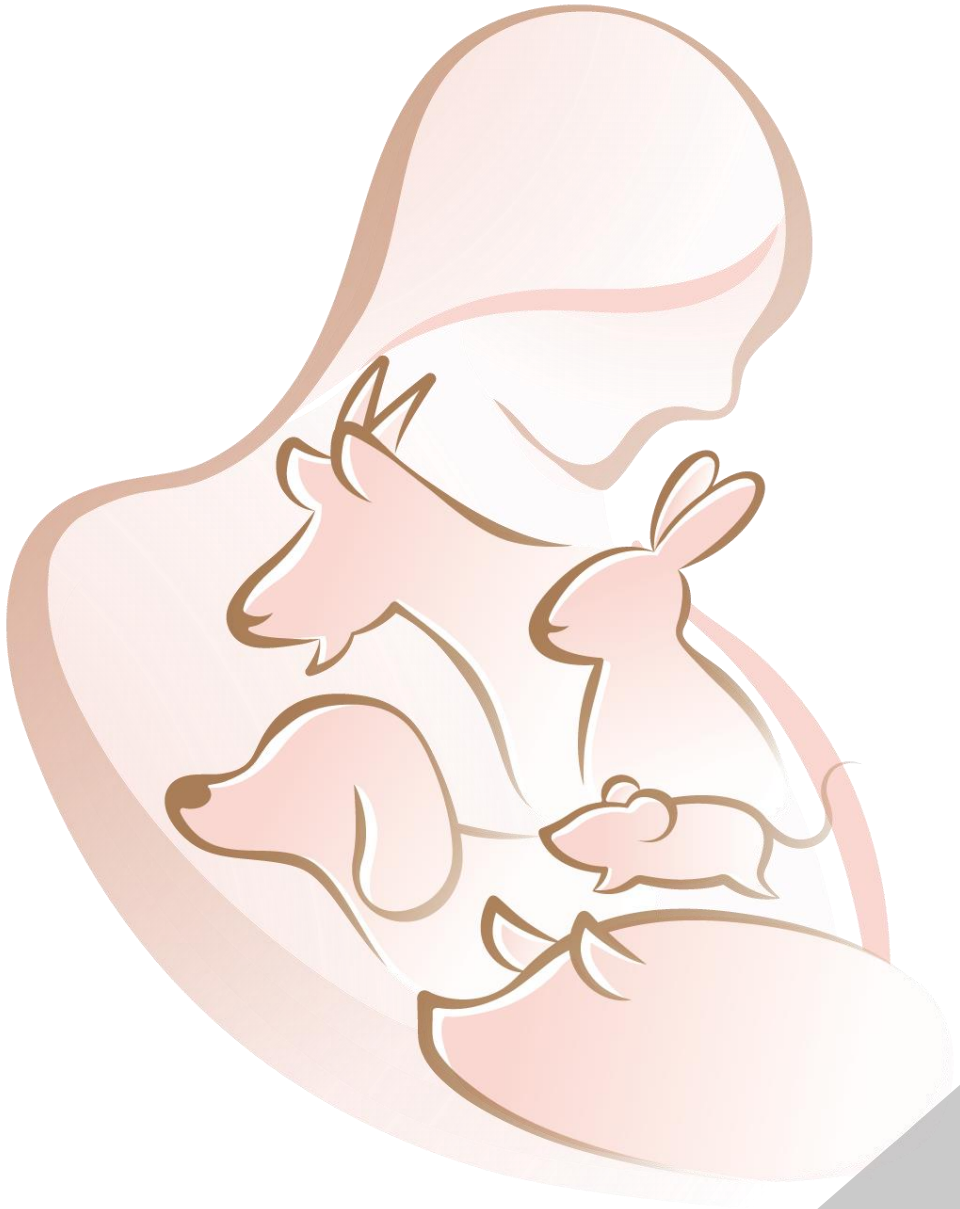
實驗數據的納入

- ✓ 最重要的實驗結果 (實驗假說)
  - ✓ 用來推估樣本數的那一項重要指標，應優先計算、納入與結論
  - ✓ 例如: 腫瘤藥效測試 vs 腫瘤抑制效果
- ✓ 次要的實驗結果
  - ✓ 列出所有依實驗規劃進行的量測結果 (細胞存活、行為、病理等)
  - ✓ 避免依故事情節挑選結果

實驗結果與討論

- ✓ 基礎值、樣本分析狀況
  - ✓ 報告絕對數值(如10/20)，而不只是處理過的數據 (如 50%)
- ✓ 結果與評估
  - ✓ 此研究可能造成偏移(bias)的任何潛在來源、所使用的動物模式的局限性
  - ✓ 研究結果是否可以應用到其他物種或其他系統，尤其是與人類醫學相關性

動物實驗



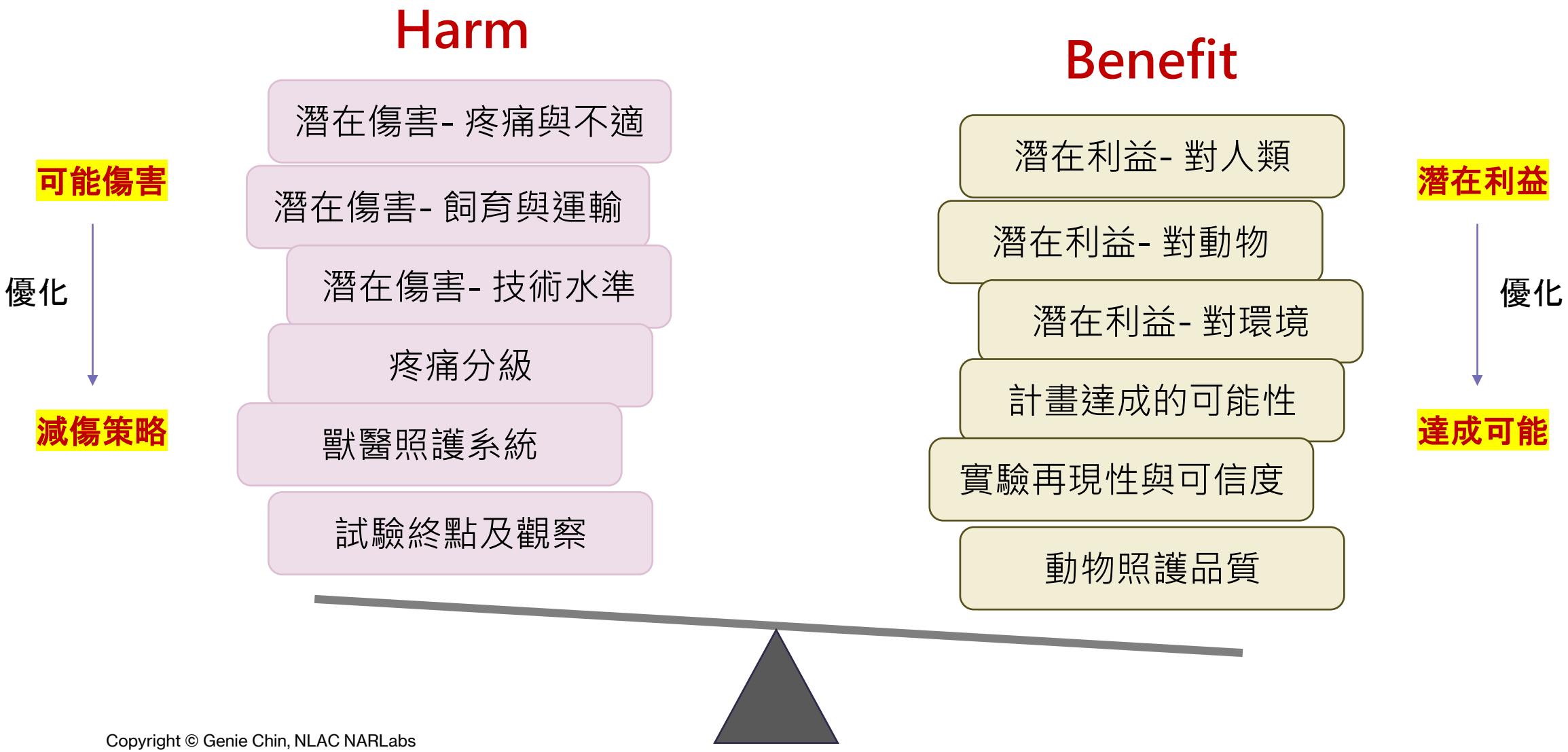
Thank You

動物實驗優化策略
傷害利益評估 Harm-Benefit Analysis

國家實驗動物中心 秦咸靜主任

NARLabs 國家實驗研究院

HARM-BENEFIT ANALYSIS



HARM ANALYSIS 傷害評估

預測可能的疼痛

- 預試驗 (pilot study)
- 風險管理
  - 危害識別：辨認出會導致疼痛的原因
  - 危害特性：描述出造成疼痛的機制
  - 曝露評估：評估曝露在疼痛下的時間及程度；
  - 風險特性：綜合評估動物感受疼痛的狀況和嚴重程度。

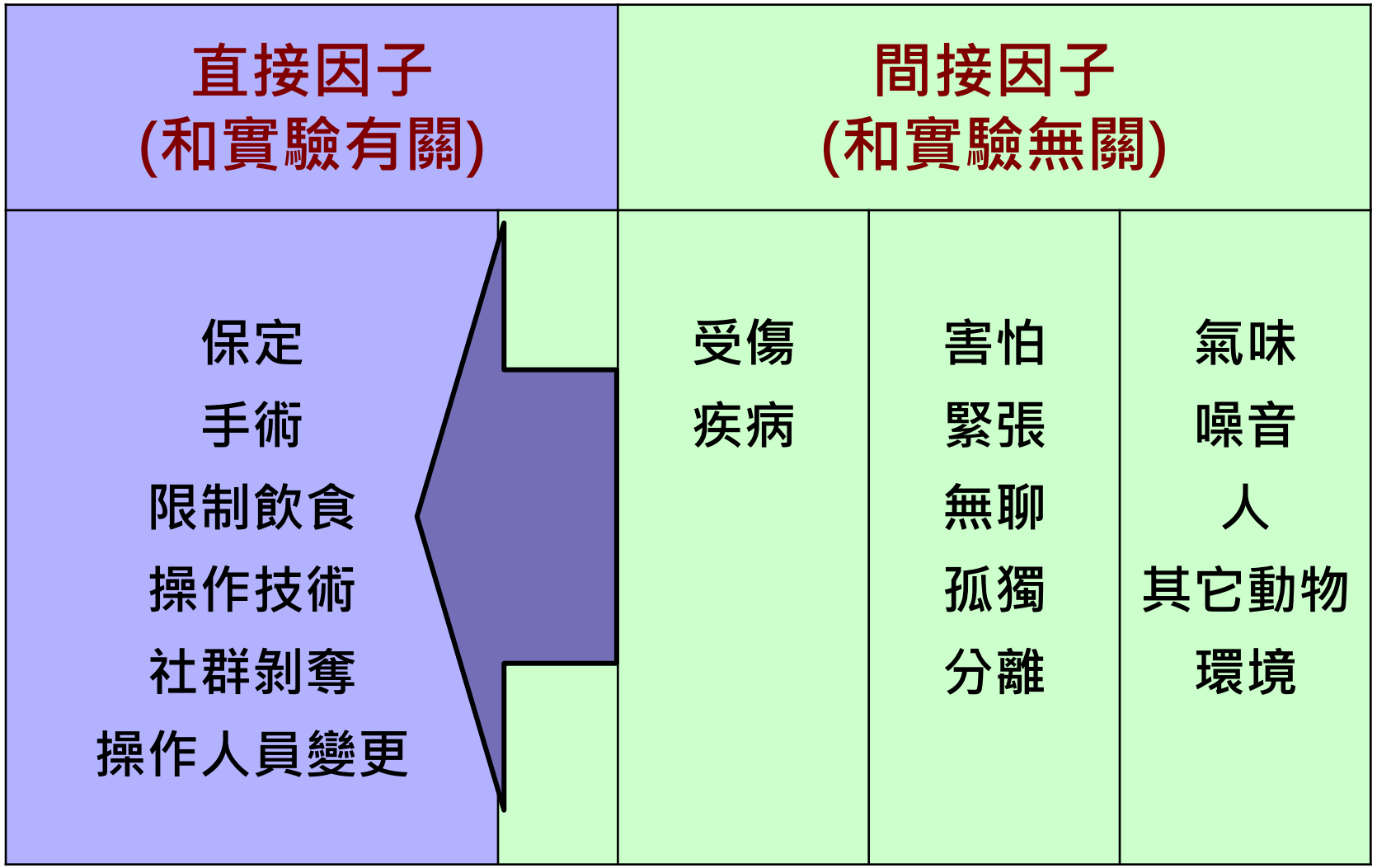
建立監控疼痛的策略

- 在實驗進行中，用來評估動物身心狀況的臨床症狀
- 用來決定何時應人道介入(例如安樂死)的臨床症狀；
- 若發現問題時，應採取的行動
- 監控的頻率；
- 負責監控的人員及其教育訓練
- 觀察記錄文件保存方式。

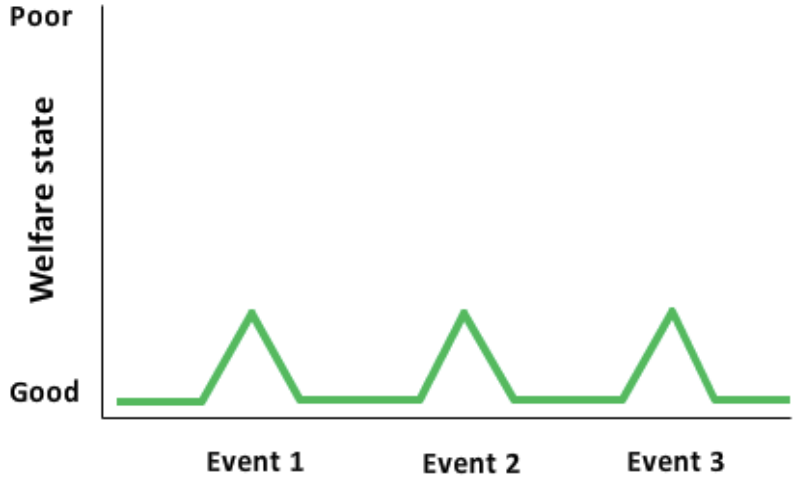
POTENTIAL STRESSOR

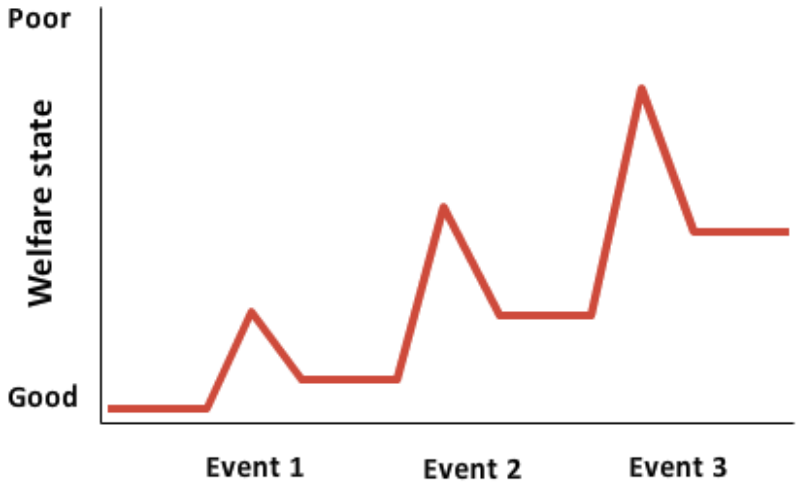
生理壓力	心理壓力	環境壓力
受傷 手術 疾病 脫水 飢餓	害怕 緊張 無聊 孤獨 分離	保定 氣味 噪音 人 其它動物 環境

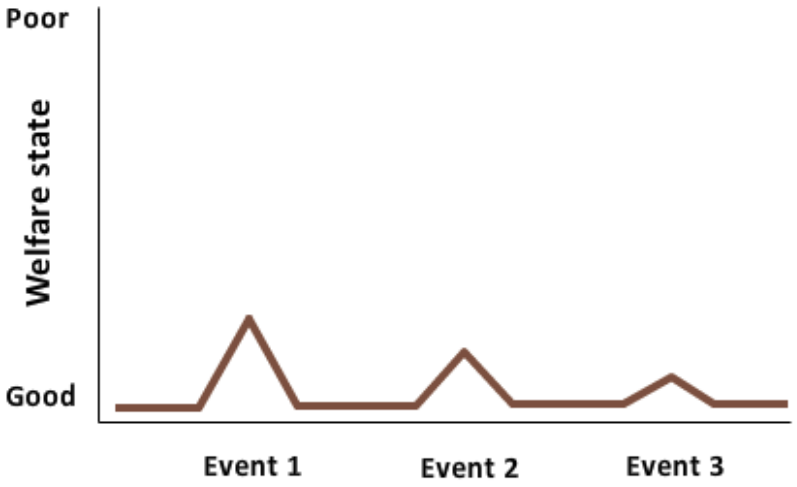
POTENTIAL STRESSOR

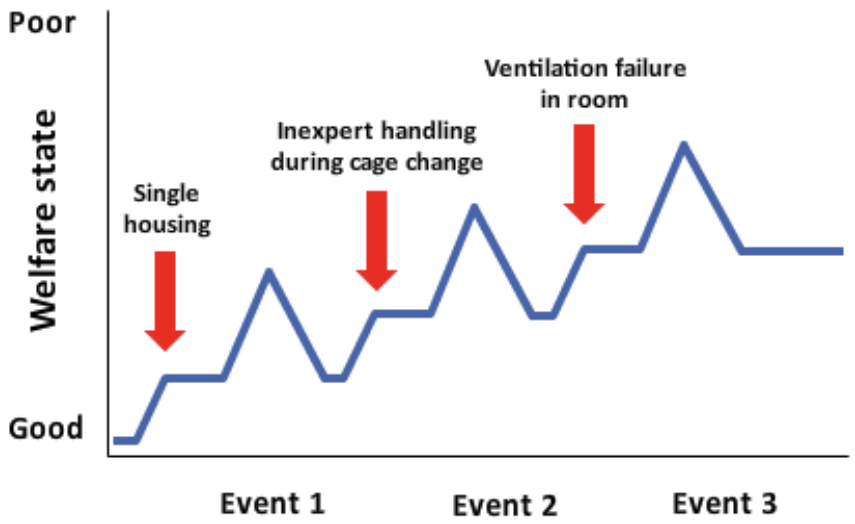


熟練的技術是動物實驗基礎









臨床觀察能力是必備之技能

籠邊觀察

1.觀察飼料及飲水

2.觀察籠邊及墊料

4. 外觀/體態/行為/步伐/活動力

開籠蓋觀察

1. 抓取動物

2. 觀察動物腹部/背部及反應

3. 觀察動物頭部、尾部、四肢

SIGNS OF PAIN 疼痛症狀

急性疼痛	慢性疼痛
減少飲食	食慾喪失、體重下降、身體狀況差
防衛 (想要保護、逃跑或咬人)	行為改變 (攻擊、無反應、過動、孤僻)
發聲 (碰到痛處會大叫)	排尿及消化功能改變
移除痛點 (舔、咬、抓、磨、搖)	自殘
不安 (換腳站、坐立難安、踏步)	流淚、紫淚
不理毛	不理毛
姿勢異常 (低頭、拱背、表情、腹部)	活動力下降 (不想動、站不起來)
流汗 (會流汗的動物)	離群獨居

52

建立臨床觀察記錄Clinical care record

P.I. _____ Protocol # _____ Date ____/____/____ Time _____

Research lab contact person(s) _____ Phone(s) _____

Building/Room # _____ Rack # _____ Shelf/Row # _____

Animal ID# _____

Animal Identification:

( ) mouse ( ) rat ( ) frog ( ) guinea pig ( ) rabbit ( ) swine ( ) sheep
( ) other _____

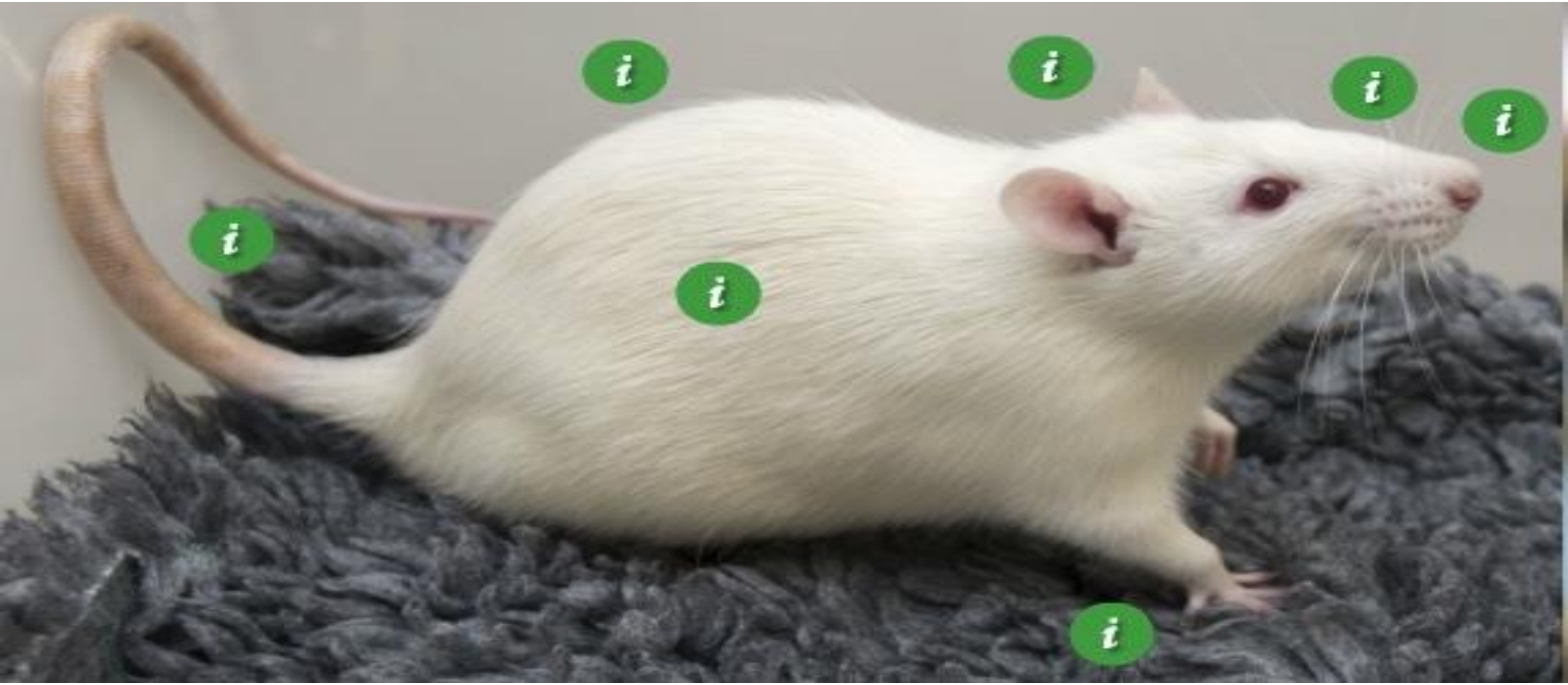
Strain/Transgenic info _____
Receipt date _____ Date of birth: _____ Sex ___M ___F Vendor/source _____

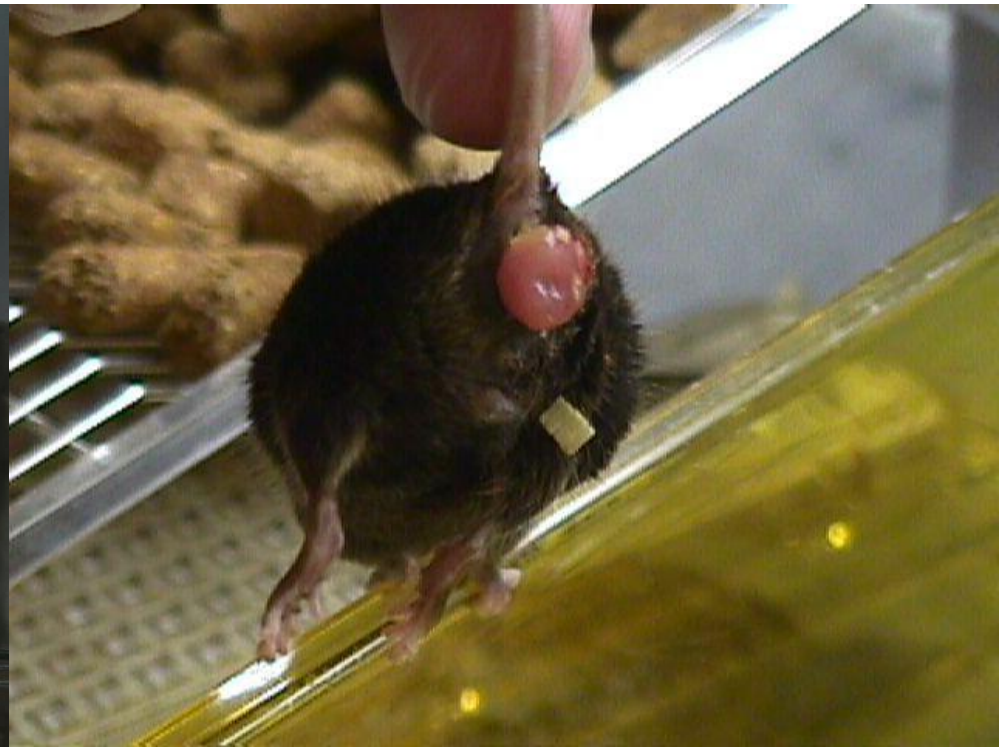
NOTE: Contact Supervisor and/or Veterinary Staff immediately if animal(s) appear in distress.

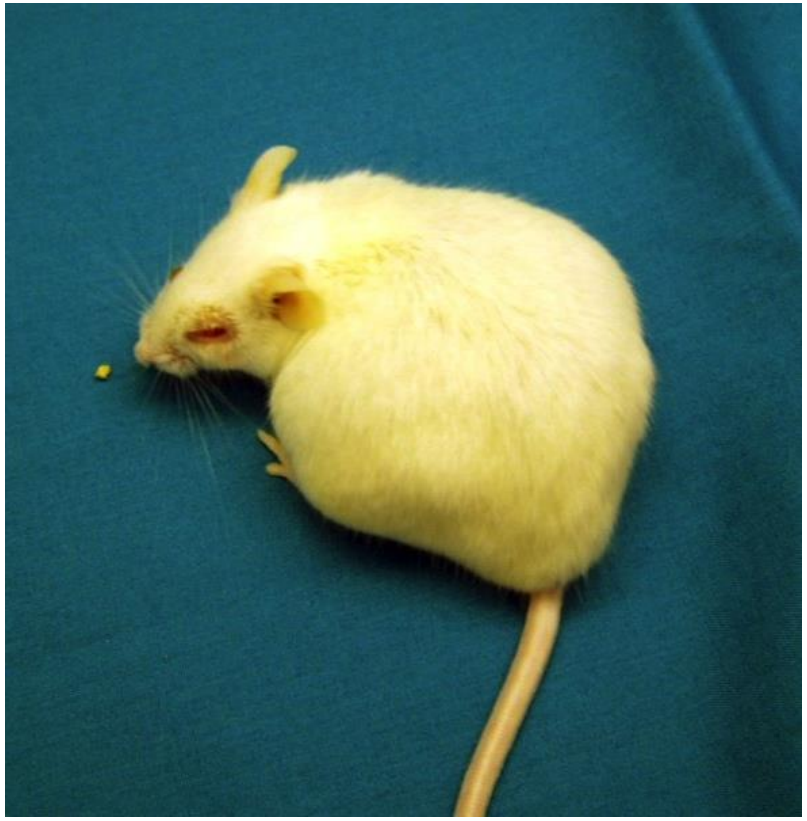
SIGNS OF ILLNESS (please check all appropriate boxes and add description as necessary):

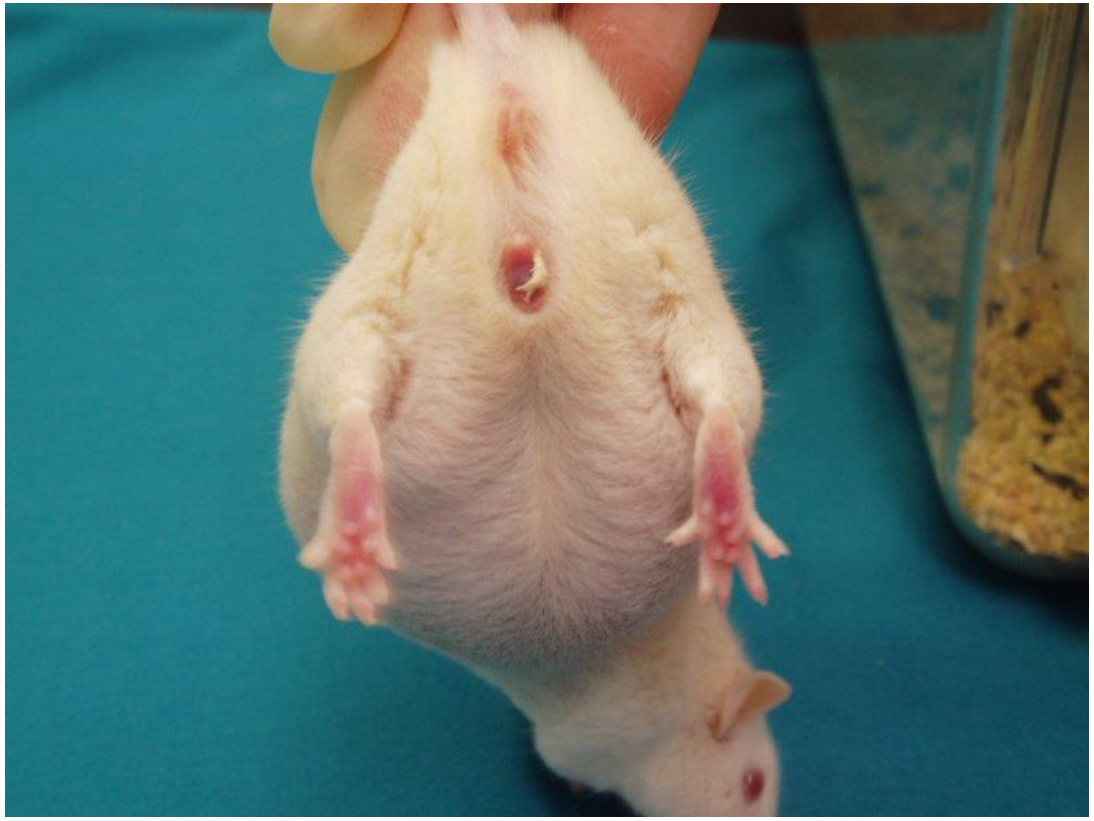
( ) hunched posture	( ) skin condition	( ) difficulty breathing	( ) not eating/drinking
( ) not responsive	( ) open wound	( ) discharge from nose	( ) malocclusion
( ) rough coat	( ) scratching	( ) sneezing	( ) diarrhea
( ) less active	( ) hair loss	( ) coughing	( ) vomiting
( ) hyperactive	( ) eye discharge	( ) fighting/aggression	( ) rectal prolapse
( ) circling	( ) mass		
( ) unsteady gait	( ) other signs observed: _____		

Animal Technician Signature _____ Date: ____/____/____









- Poor body condition
- Poor coat condition
- Poor body posture
- Moving abnormally
- Behaving abnormally

- Appearance of the eyes
- Appearance of the ears
- Appearance of the nose
- Appearance of the tail

- Poor body condition
- Poor coat condition
- Poor body posture
- Moving abnormally
- Behaving abnormally

- Appearance of the eyes
- Appearance of the ears
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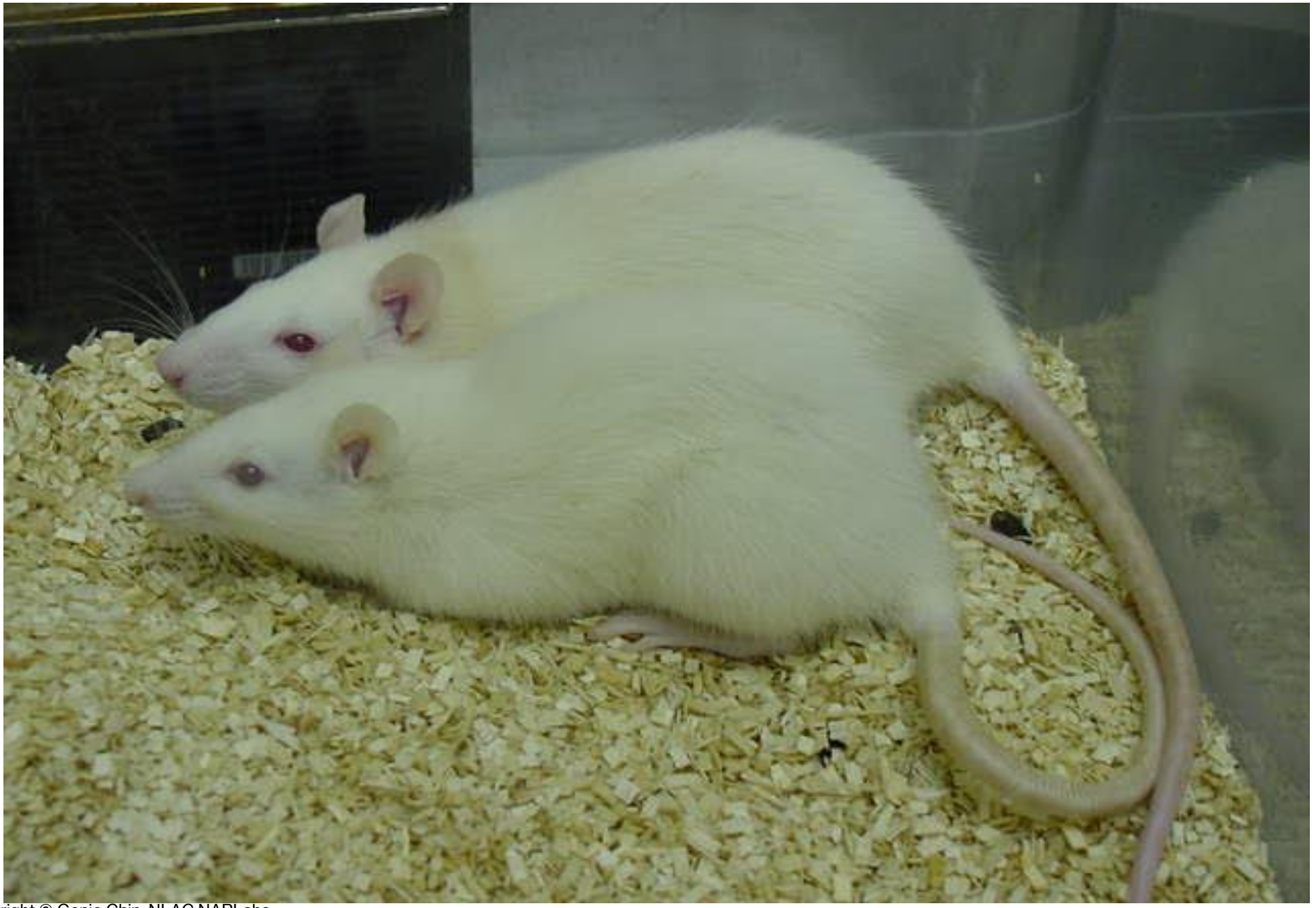
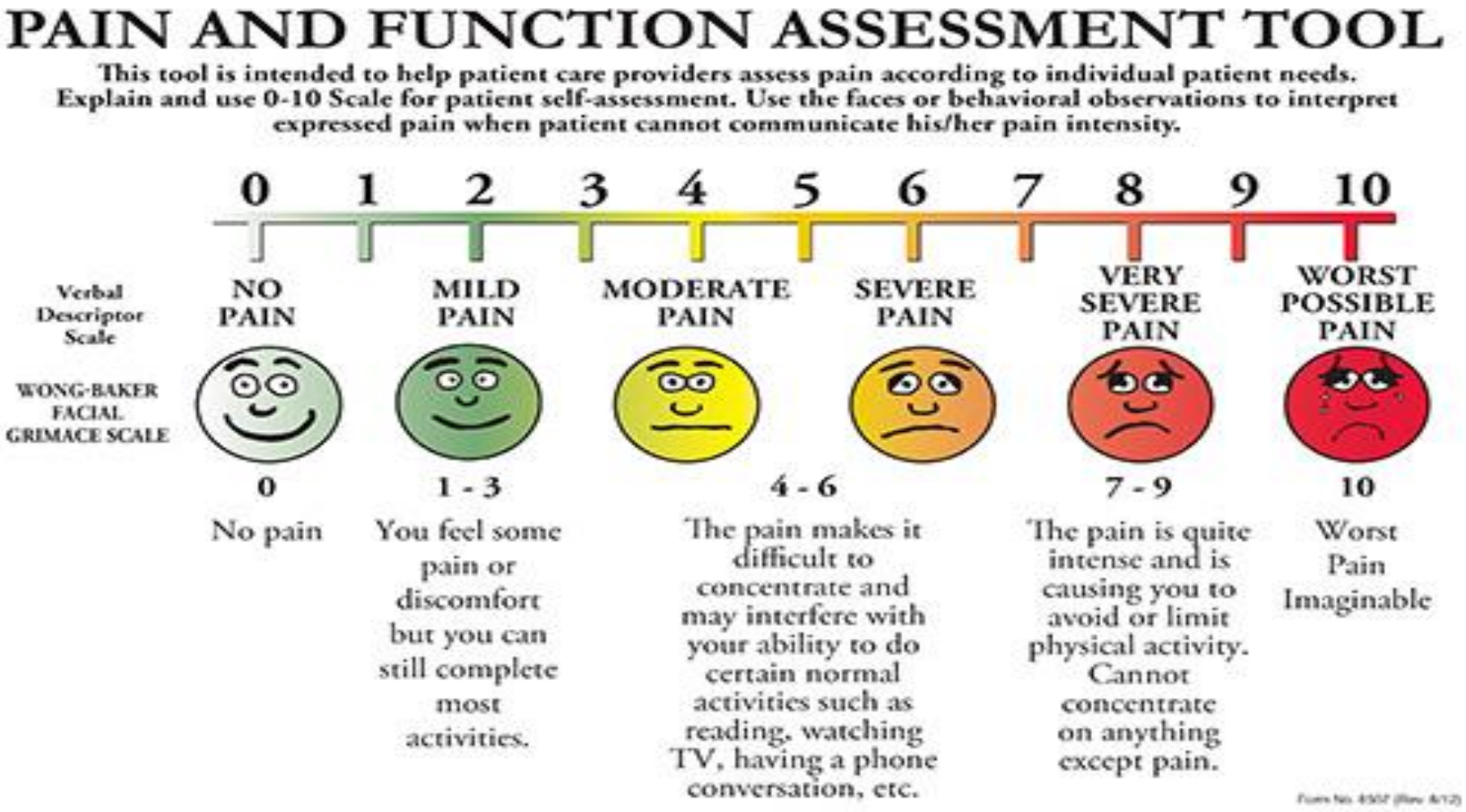
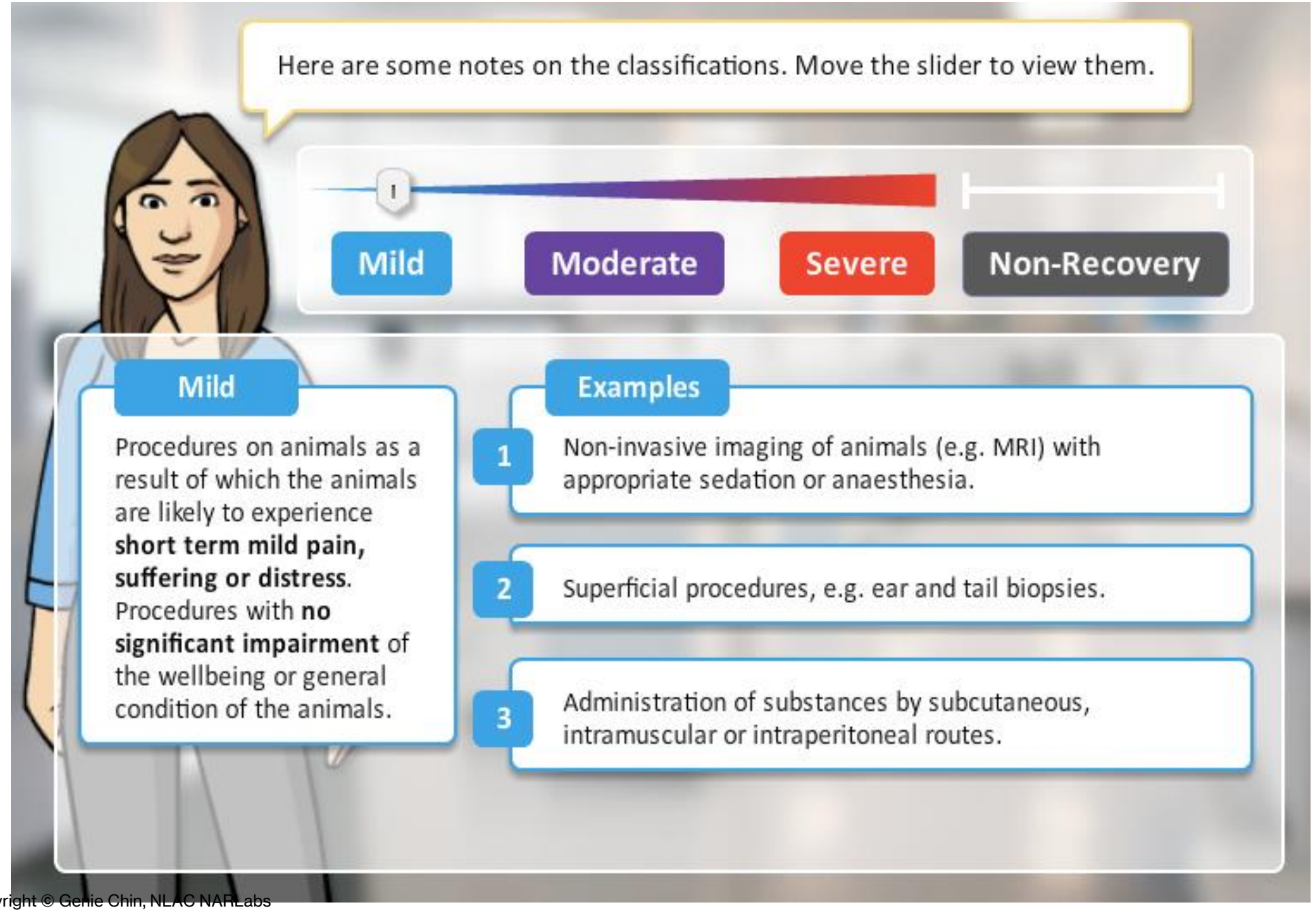
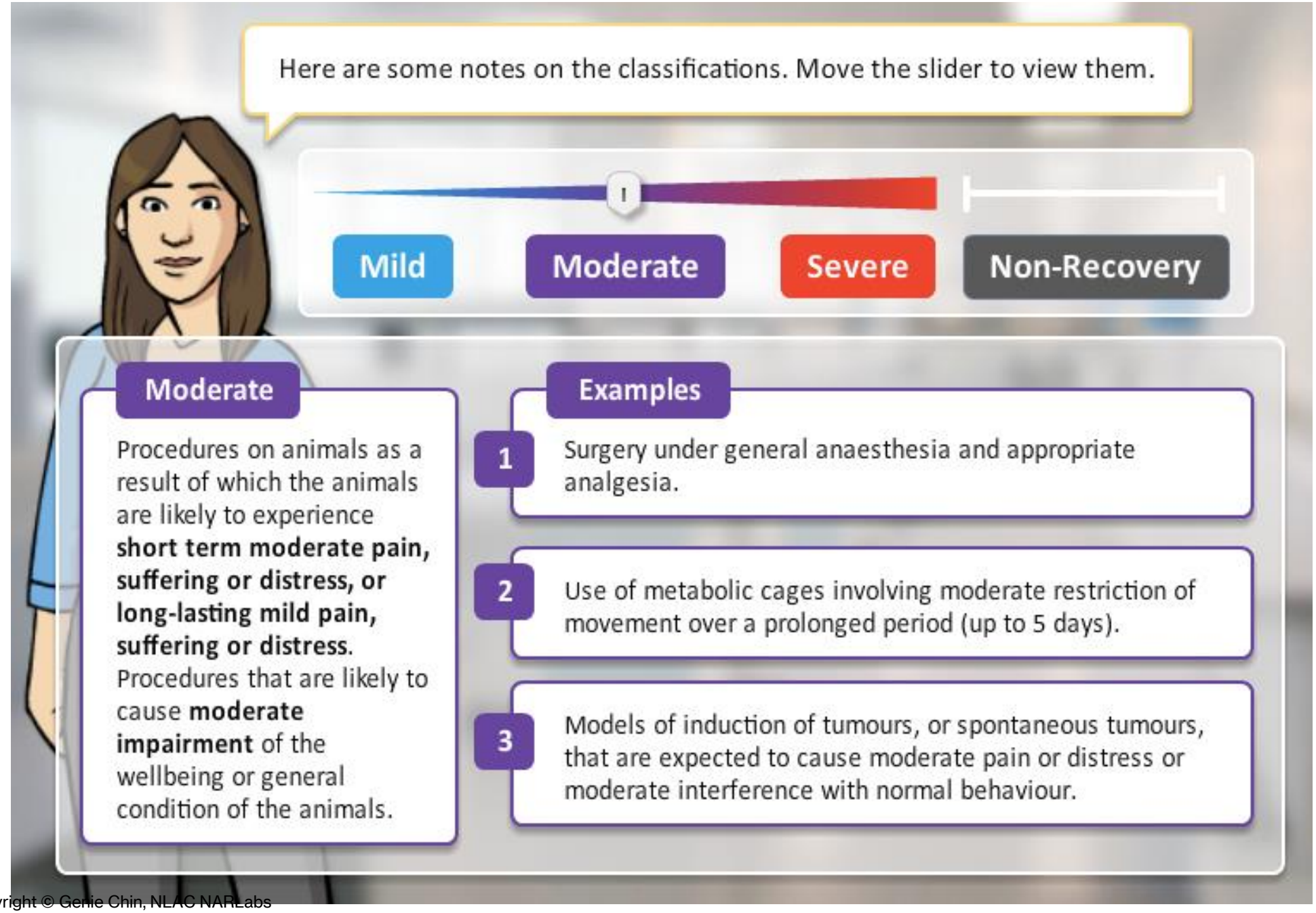
- Appearance of the tail

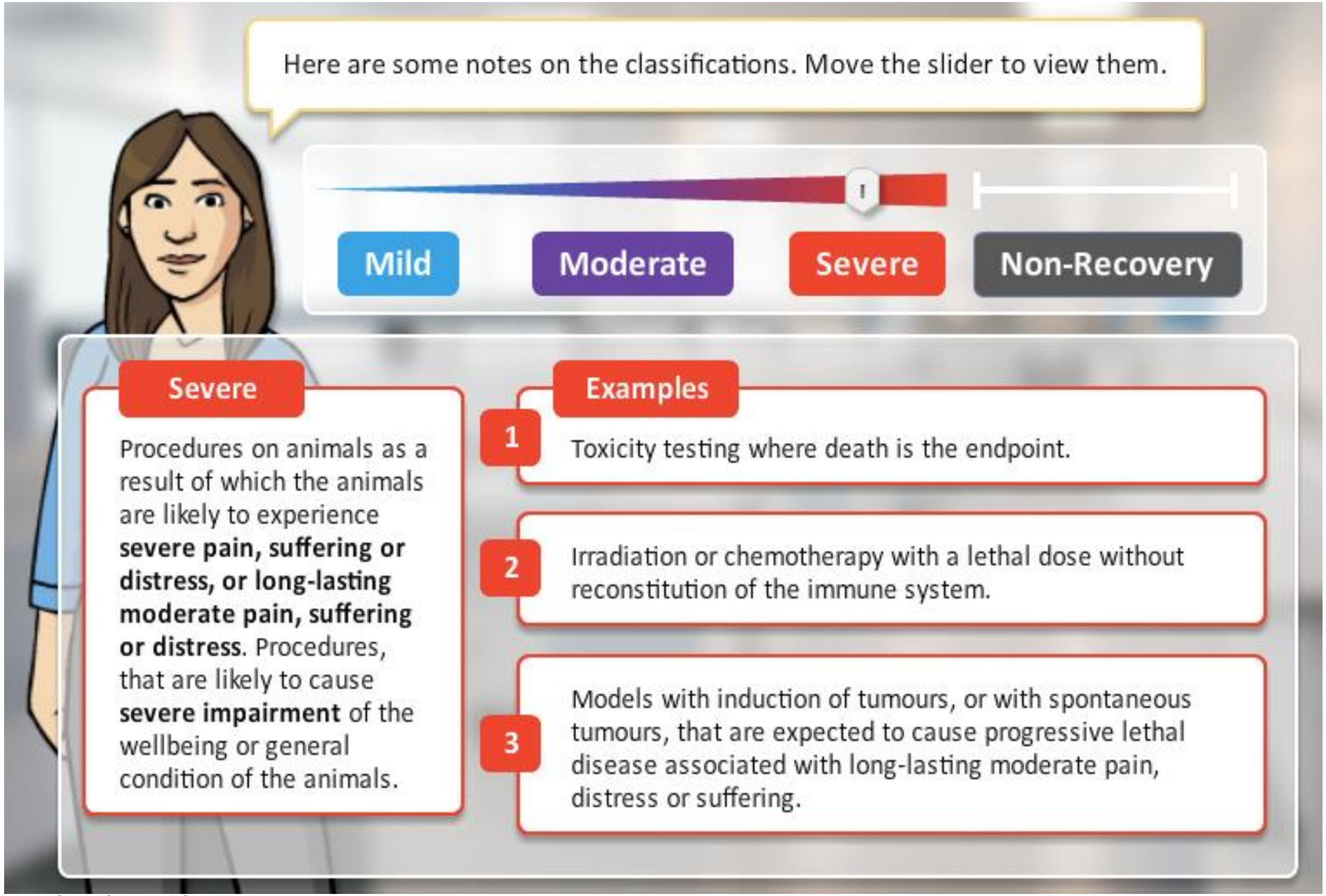


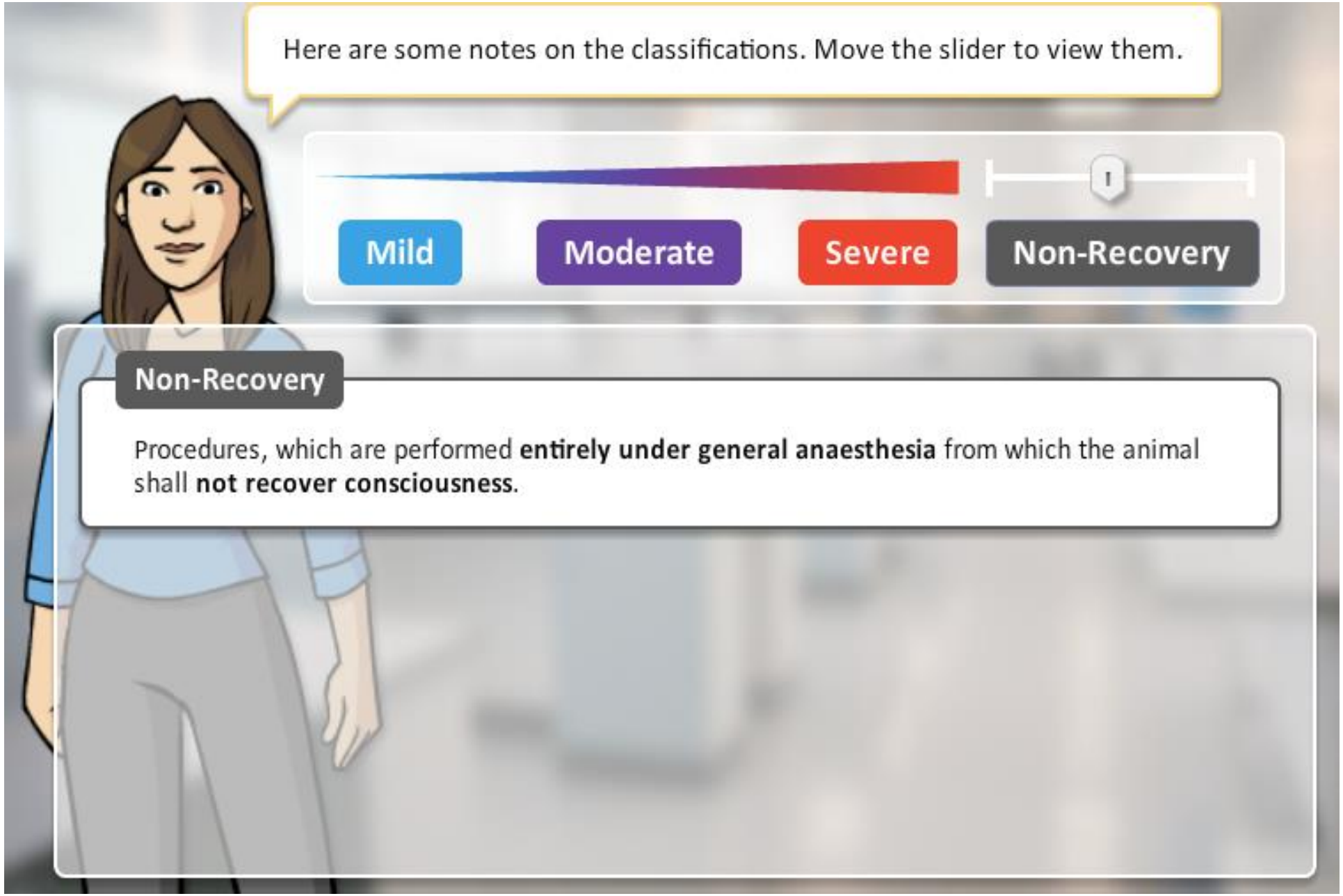
疼痛分級評估











建立臨床觀察評分表

肝病臨床觀察評分表

Characteristic	Score			
	1	2	3	4
Coat - general	Normal	Slight lack of grooming	Starey	-
Skin tone	Normal	Mildly dehydrated	Moderately dehydrated	Severely dehydrated
Behaviour	Normal	Slightly dull or lethargic	Aggressive or apathetic and inactive	Very aggressive or immobile and unresponsive
Abdominal distension	None	Mild ascites	Obvious ascites	-
Jaundice	None	Slightly jaundiced appearance	Mild jaundice present	Moderate jaundice present
Body weight	Normal weight gain or weight loss less than 5%	Weight loss <=10%	Weight loss <=15%	Weight loss <=20%

1. **挑選指標**
   - 一般性指標
   - 實驗的特殊指標
2. **決定評量模式**
   - 0-4分
   - 0-10分
   - Yes/No
   - 量測數值
3. **決定分數級距的意義**
   - 0-6 正常
   - 7-9 輕微，多觀察
   - 10-14 介入治療
   - 15-22 人道安樂死

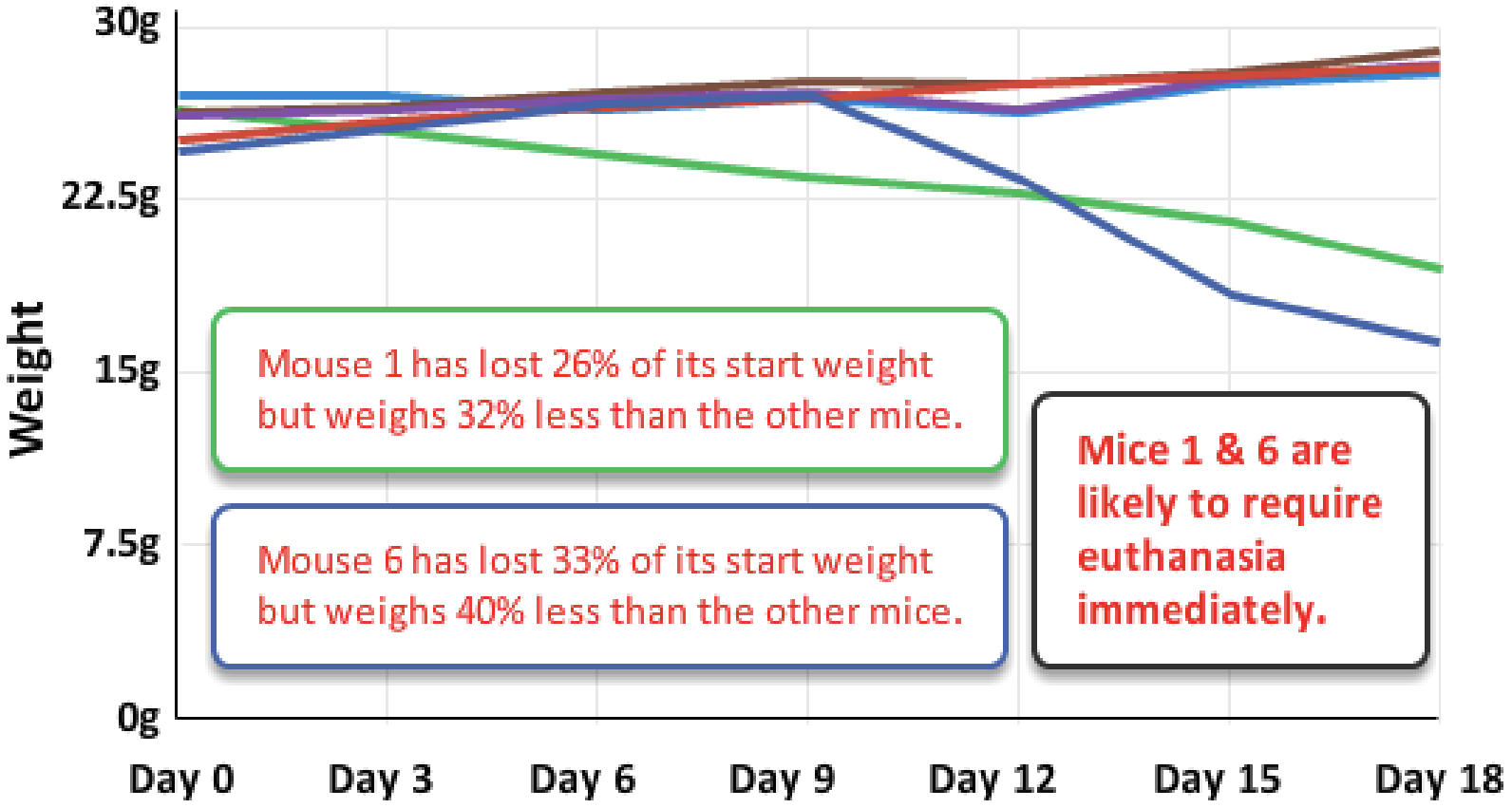
觀察項目	0	1	2	3
行為				
姿態				
呼吸				
毛髮狀況				
眼睛				
身體狀況				

觀察項目	0	1	2	3
行為				
姿態				
呼吸				
毛髮狀況				
眼睛				
身體狀況				

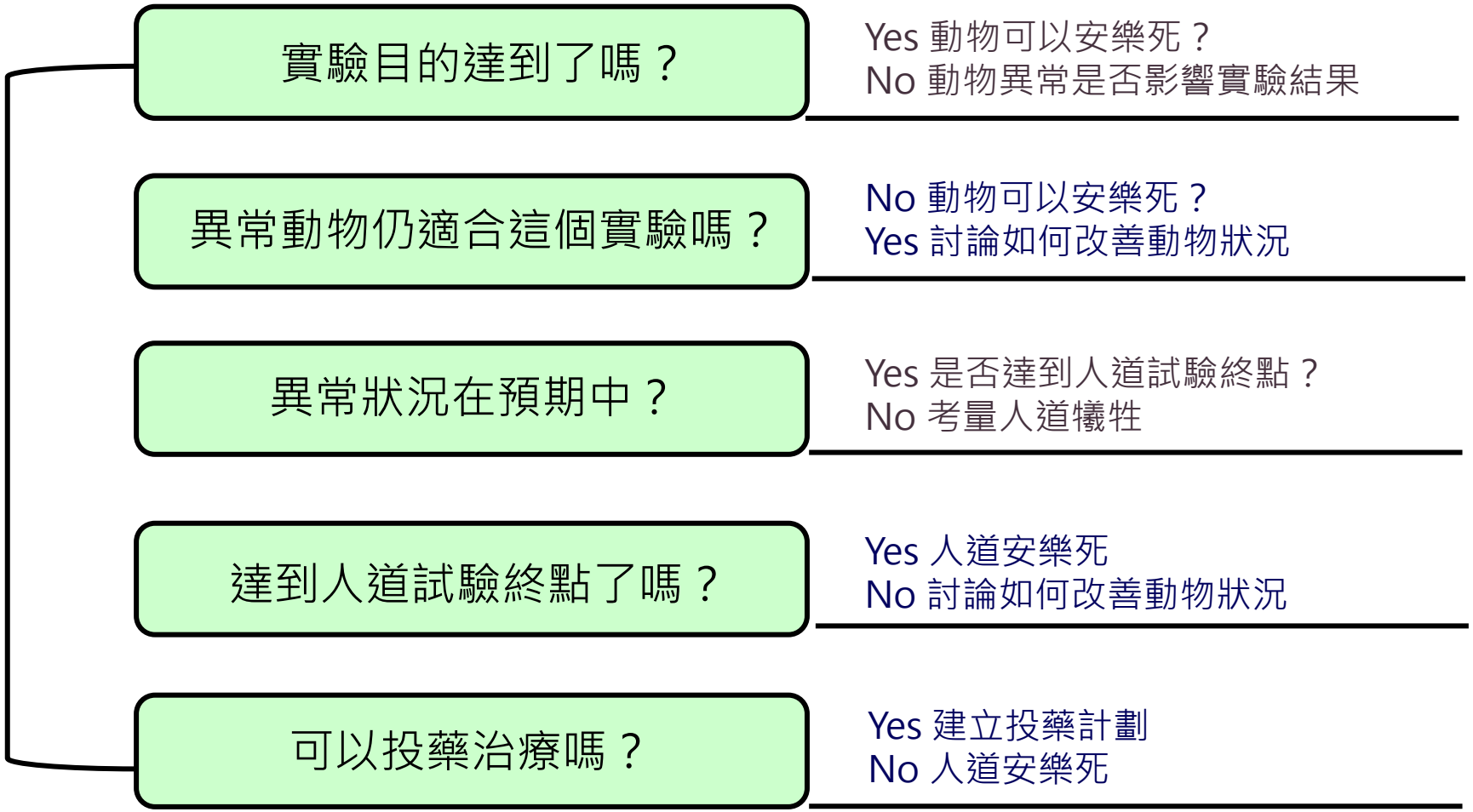
腫瘤臨床觀察評分表

Characteristic	Score			
	1	2	3	4
Coat - general	Normal	Slight lack of grooming	Starey	-
Skin tone	Normal	Mildly dehydrated	Moderately dehydrated	Severely dehydrated
Behaviour	Normal	Slightly dull or lethargic	Apathetic and inactive	Immobile and unresponsive (for tumour interfering with normal behaviour and activities
Tumour size and appearance	Very small (<3mm)	Tumour small (<=5mm) with no necrosis or ulceration	Tumour intermediate size (<=8mm) with no necrosis or ulceration	Tumour large (>=12mm) Ulceration of tumour of any size
Body weight	Normal weight gain or weight loss less than 5%	Weight loss <=10%	Weight loss <=15%	Weight loss <=20%

體重變化

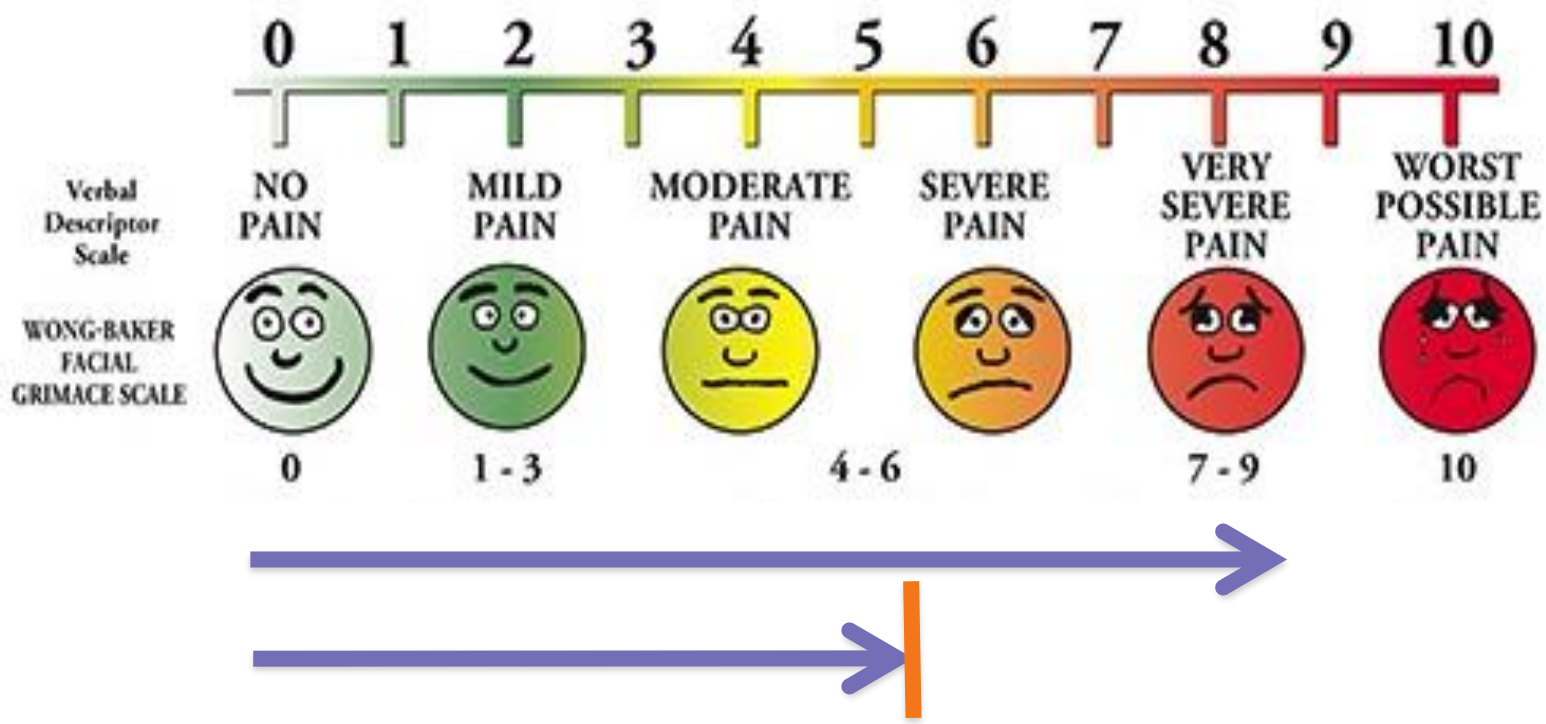


發現異常動物之流程

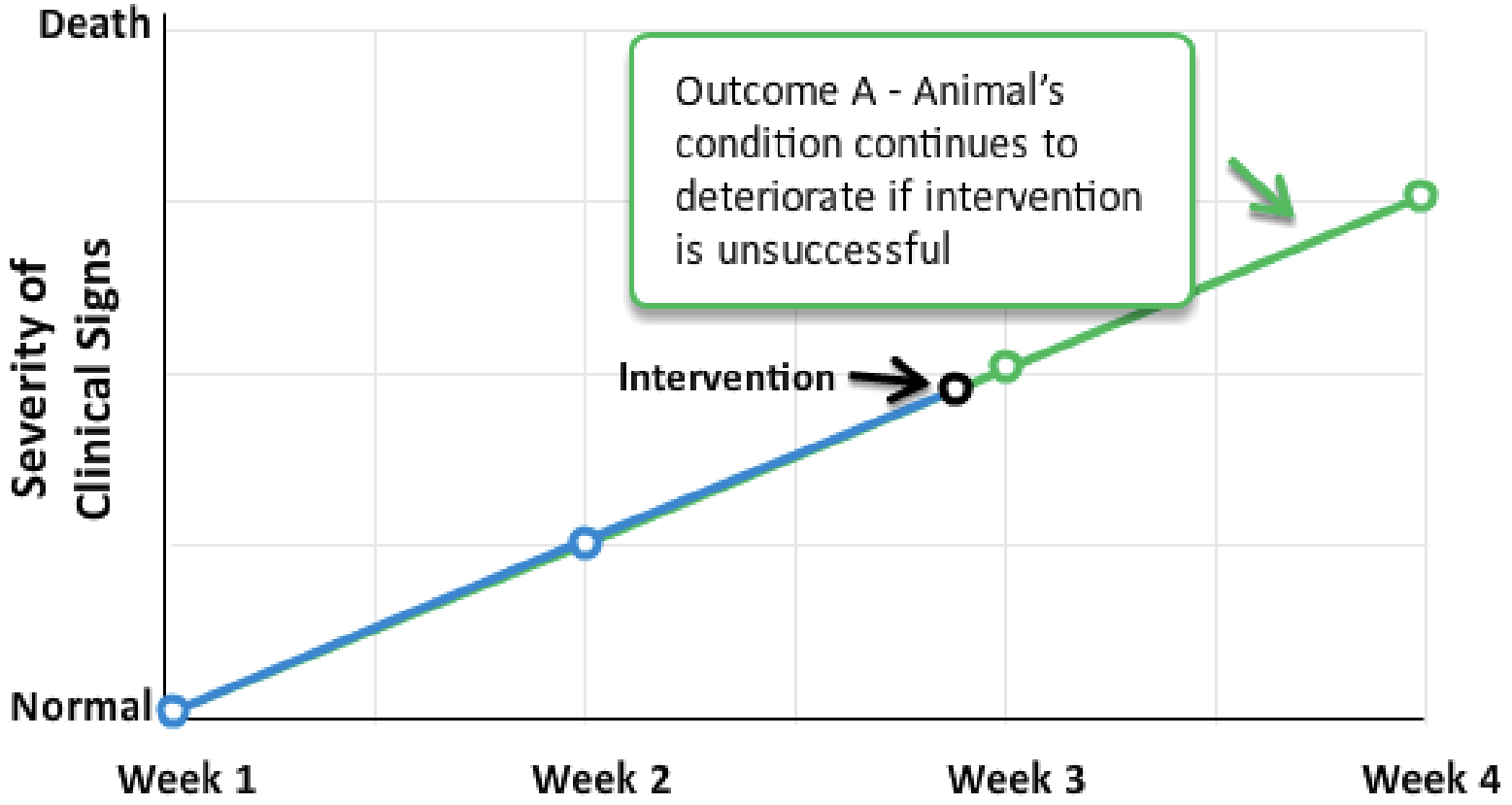


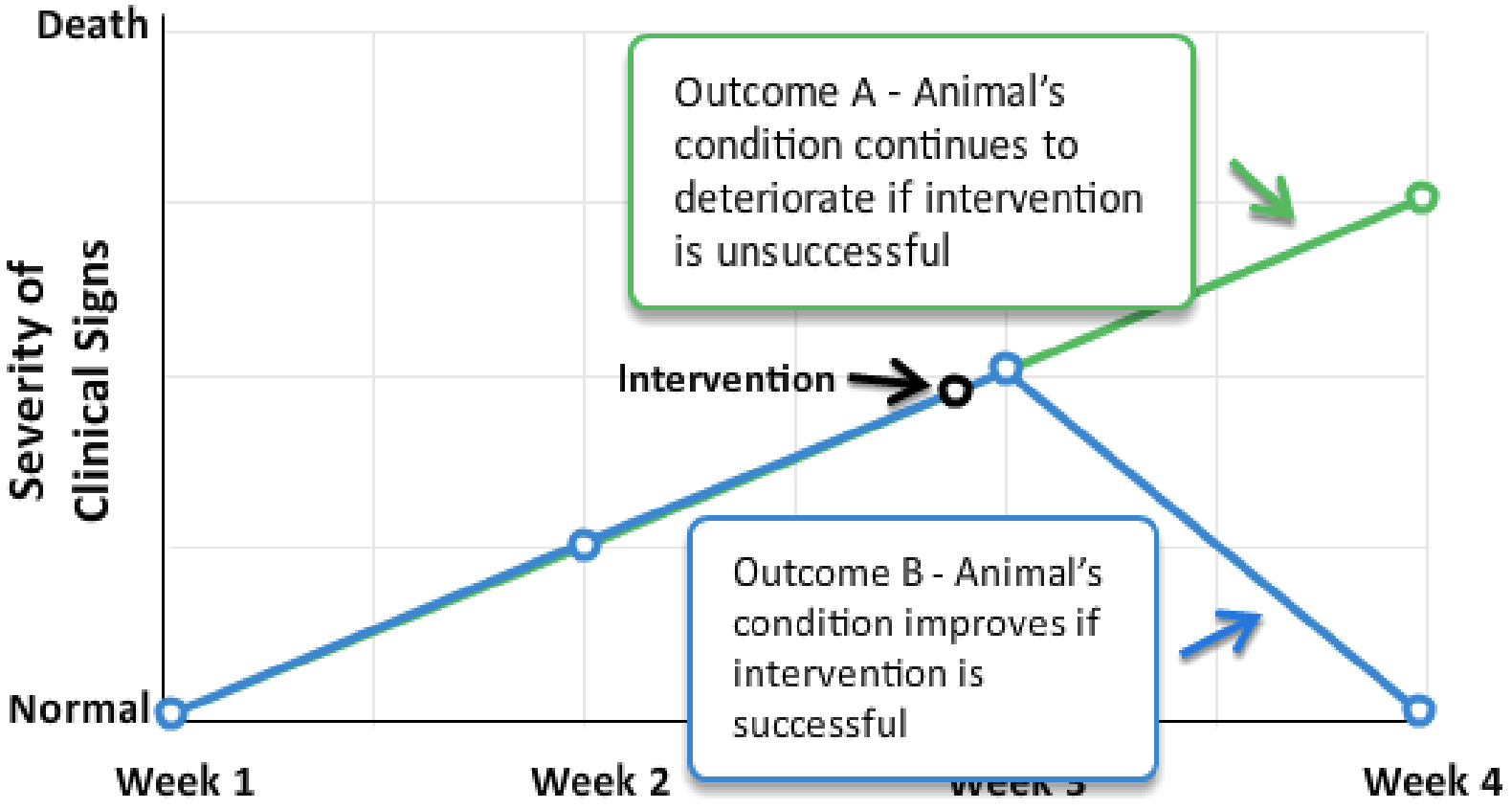
人道介入

- 實驗取得到足夠的資料，可結束實驗
- 動物可忍受疼痛程度的上限

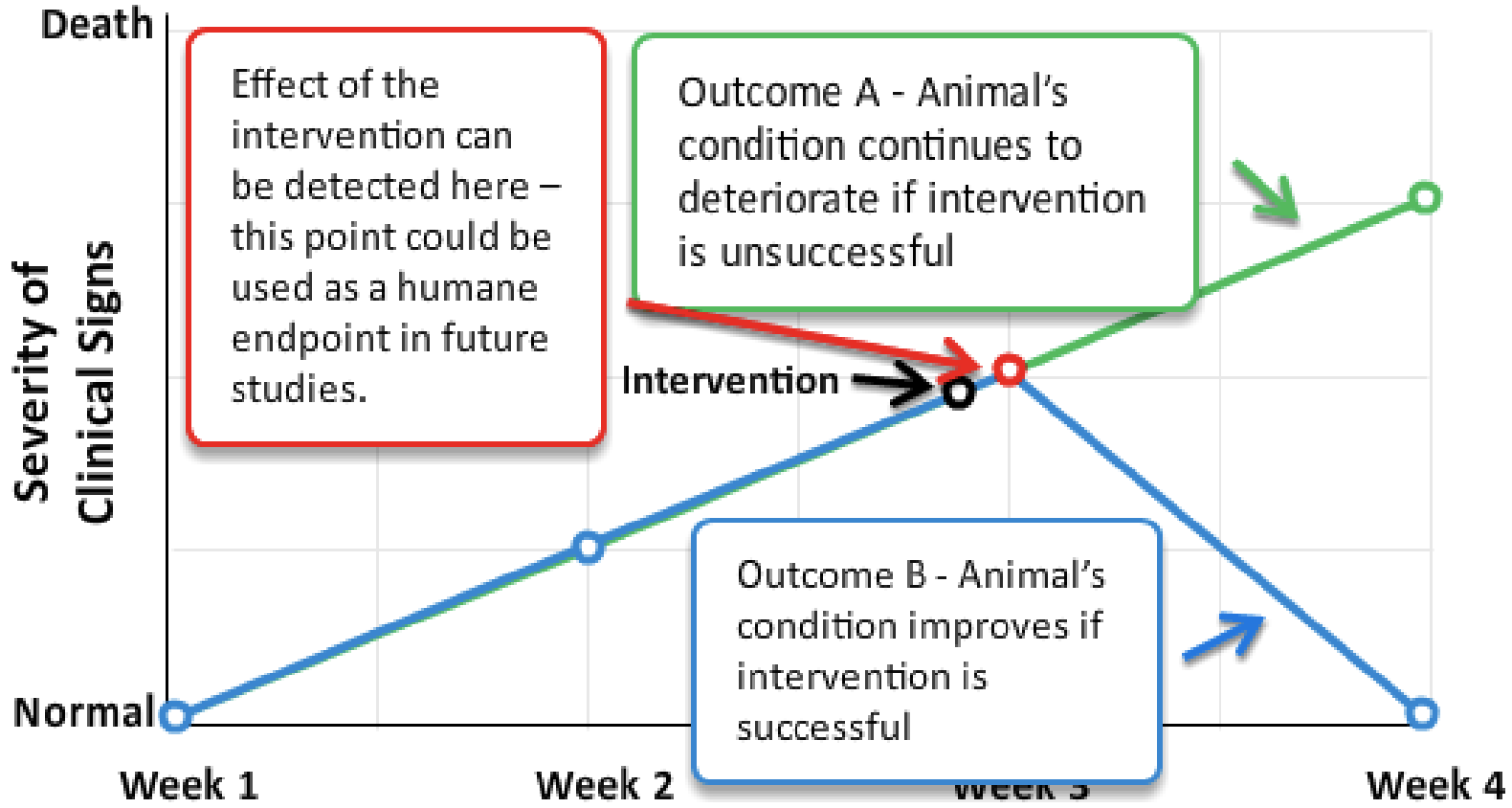


人道介入

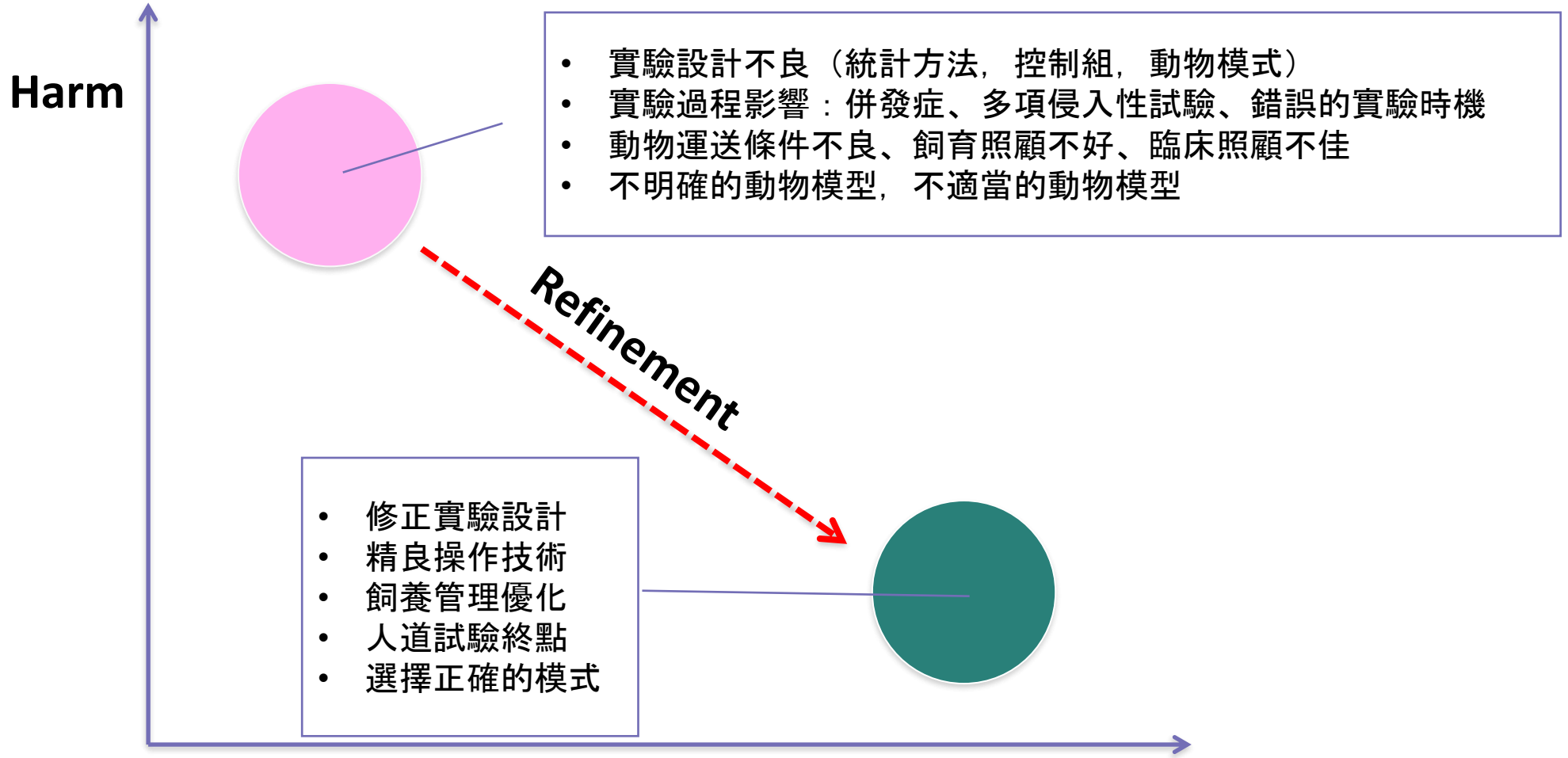




人道試驗終點



透過HBA進行實驗優化



BENEFIT ANALYSIS 利益評估

實驗所宣稱會達到的效益

若是無法評量，等於達不到

- 這個研究有助於癌症醫學發展...
- 這個研究有助於了解A藥物是否可作用於B，有助於抑制腫瘤的血管新生

若是做不到，等於達不到

- 這個實驗團隊從來沒做過
- 這個學校的動物房沒有管理機制

執行一個可信的動物實驗

繁殖育種
環境豐富化
健康品質
社群行為

動物資源
供應單位

動物運輸

動物實驗
執行單位

檢疫及適應
環境豐富化？
健康品質？
社群行為？

動物實驗

實驗設計

正確動物？
飼料及營養？
分組及統計？
飼育環境？

實驗操作
採樣分析

保定技術？
投藥方式？
採樣位置？
實驗及病理分析？

PREPARE

norecopa

The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith[a], R. Eddie Clutton[b], Elliot Lilley[a], Kristine E. Aa. Hansen[c] & Trond Brattelid[a]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; [d]Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; [e]Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

PREPARE[1] consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE[2].
PREPARE covers the three broad areas which determine the quality of the preparation for animal studies:

1. **Formulation of the study**
2. **Dialogue between scientists and the animal facility**
3. **Quality control of the components in the study**

The topics will not always be addressed in the order in which they are presented here, and some topics overlap. The PREPARE checklist can be adapted to meet special needs, such as field studies. PREPARE includes guidance on the management of animal facilities, since in-house experiments are dependent upon their quality. The full version of the guidelines is available on the Norecopa website, with links to global resources, at **https://norecopa.no/PREPARE**.
The PREPARE guidelines are a dynamic set which will evolve as more species- and situation-specific guidelines are produced, and as best practice within Laboratory Animal Science progresses.

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. ☐ Assess the reproducibility and translatability of the project.
2. Legal issues	☐ Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. ☐ Locate relevant guidance documents (e.g. EU guidance on project evaluation).
3. Ethical issues, Harm-Benefit Assessment and humane endpoints	☐ Construct a lay summary. ☐ In dialogue with ethics committees, consider whether statements about this type of research have already been produced. ☐ Address the 3Rs (Replacement, Reduction, Refinement) and the 3Ss (Good Science, Good Sense, Good Sensibilities). ☐ Consider pre-registration and the publication of negative results. ☐ Perform a Harm-Benefit Assessment and justify any likely animal harm. ☐ Discuss the learning objectives, if the animal use is for educational or training purposes. ☐ Allocate a severity classification to the project. ☐ Define objective, easily measurable and unequivocal humane endpoints. ☐ Discuss the justification, if any, for death as an end-point.
4. Experimental design and statistical analysis	☐ Consider pilot studies, statistical power and significance levels. ☐ Define the experimental unit and decide upon animal numbers. ☐ Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.

ARRIVE

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

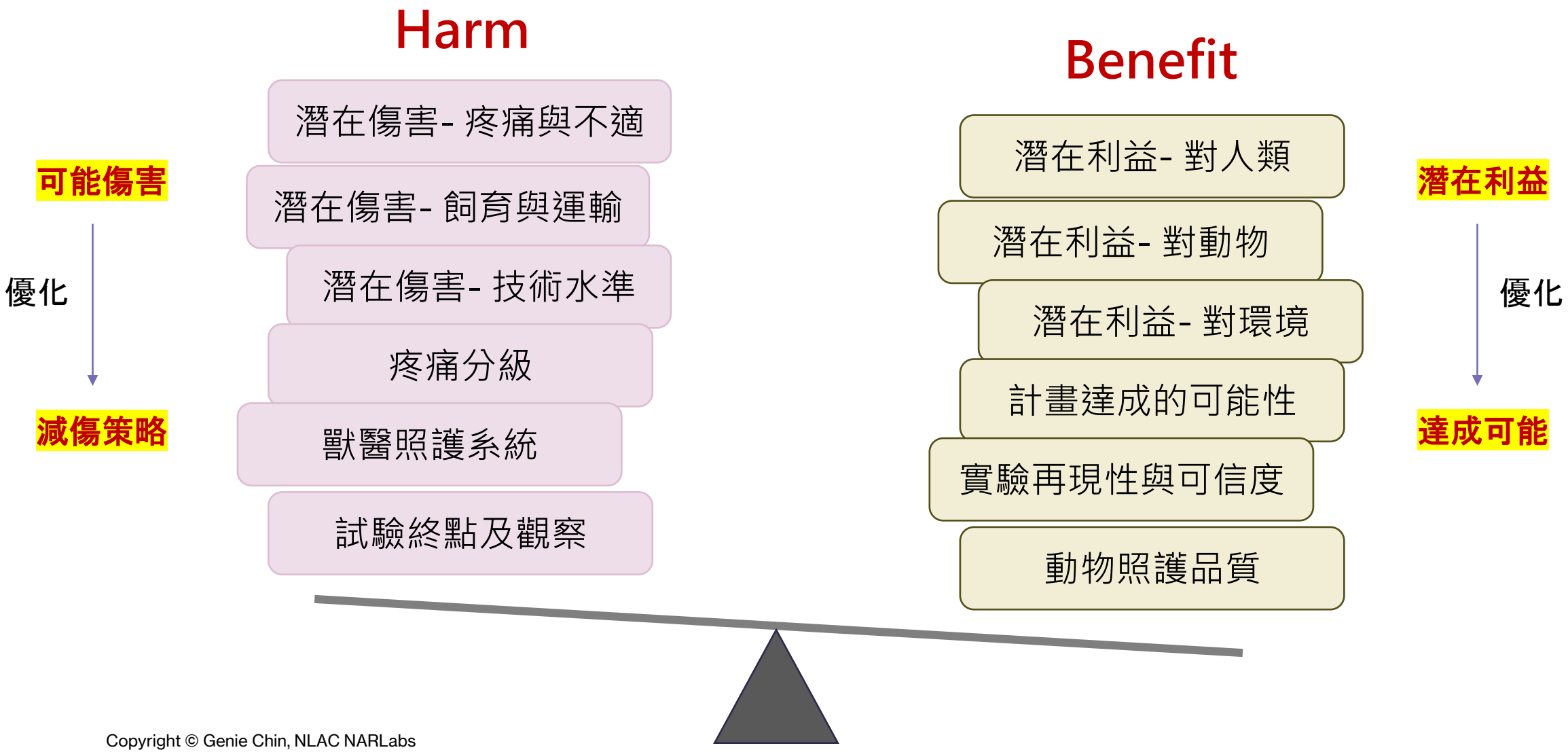
Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

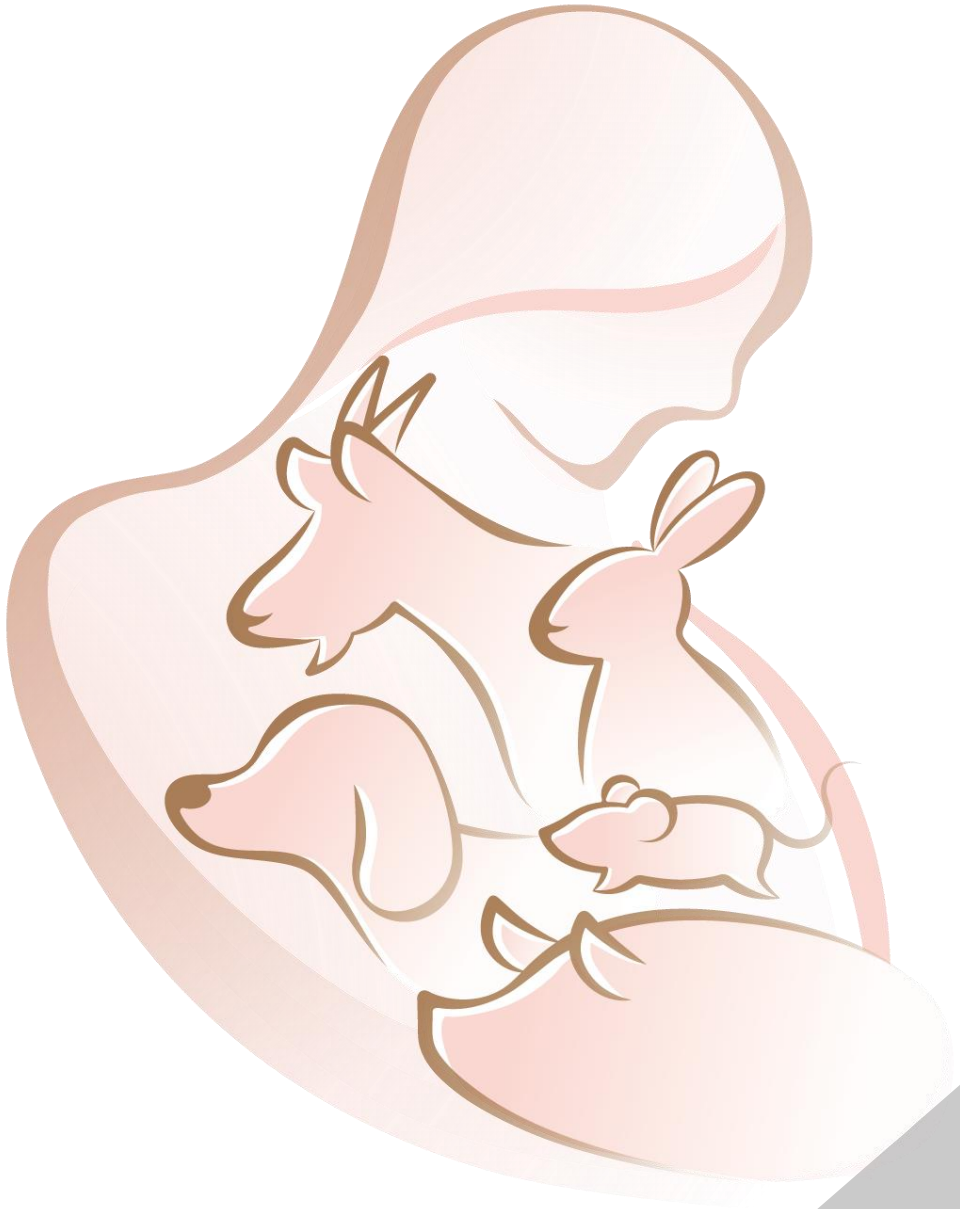
[1]The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK, [2]School of Veterinary Science, University of Bristol, Bristol, UK, [3]School of Biological Sciences, University of Bristol, Bristol, UK, [4]National Heart and Lung Institute, Imperial College London, UK, [5]Centre for Statistics in Medicine, University of Oxford, Oxford, UK.

	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	

The ARRIVE guidelines. Originally published in *PLoS Biology*, June 2010[2]

HARM-BENEFIT ANALYSIS





Thank You