

# **Animals in Research**

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# What is an Animal Experiment?

- This is a multidisciplinary process which involves using animals for research
- It is useful to solve specific problems in medical, dental, biomedical or veterinary sciences
- Adequate training prior to handling animals and adequate justification of the animal experiment is essential

# Early History of Animal Experiments

## **Aristotle (384-322 B.C):**

Made dissections & revealed internal differences in animals.

## **Erasistratus ( 304-258 B.C) :**

Experimented on pigs & demonstrated that trachea was the air tube and lungs were pneumatic organs.

# Early History of Animal experiments.

- **Galen (130-200 AD):**

Performed anatomical dissections of pigs, monkeys and many other species.

Anatomical dissections of dead animals and people had been among the earliest types of experimentation in medieval time and it was prohibited.

**continued.....**

# Modern History of Animal Experiments.

- There was reawakening of interest in science in 1500
- **Andres Vaseilius (1514-1564) :**  
Founder of modern anatomy and demonstrated dog and pig anatomy in public
- **Sir Wiliam Harvey:**  
Published his work on the movement of heart and blood in animals



## **Claude Bernard**

**is known as the prince of vivisections and father of Physiology.**

**His wife**

**Marie Françoise Martin, founded the first anti-vivisection society in France in 1883**

# Categorization of animal experiments (Research)

- There are two kinds of animal experiments

## **Non infectious animal experiments.**

eg: Nutritional trails, research on non communicable diseases etc.

## **Infectious animal experiments.**

eg: Rabies, Leptospirosis, Influenza and other zoonoses etc.

Infectious experiments can be further categorized according to the severity of the infection.

# Infectious Animal Experiments





# Infectious Animal Experiments continued ---

- It should be carried out in a separate area specially designed to perform that kind of investigations
- **P1, P2, P3, P4** categories have been identified depending on the severity of the infection
- According to the virulence of the organism advanced facilities should be available
- All the animals will be euthanized at the end

# Infectious Animal Experiments continued ---

- Carcass disposal method and disinfection of cages etc. should be mentioned in the protocol
- **P4** level infectious experiments should only be carried out by a **qualified professional** in the relevant discipline



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Doing the Right Thing

# Ethics

ards

Values

Fairness

y to

# What is Ethics

What (not to) do?

How (not to ) live?

**What ( not to) approve**

# Ethical Concepts

There are three common views on animal experiments

- **1. Deontological approach:** Animals have no value and one can use them in any manner necessary
- **2. Animal rights view:** Animals have same rights as human beings. This is an activist point of view
- **3. Utilitarian view:** One can use animals for benefit of human welfare with minimum violation of animal welfare

# Five Freedom in Animal Welfare

## The Five Freedoms

for animals

Freedom to express  
normal behaviour

Freedom from  
hunger and thirst

Freedom from  
discomfort

Freedom from  
pain,  
injury or disease

Freedom from  
fear and distress



Don't  
bank  
on  
it!

Bentley

# Animal Welfare

- Animal welfare can be introduced as reducing pain and suffering in animals
- Researchers should be sensitive to understand that all procedures that would cause pain in human beings can induce pain in animals, unless specific evidence to the contrary is available
- It is important to recognize fear, pain and distress in each species of animals and this is very variable

# Ethical issues

- Regardless of the species they should be treated humanely
- In procedures that may cause pain, distress or discomfort measures to be taken to minimize pain
- Use of anaesthesia and analgesia where appropriate
- Experiment must be carried out in shortest possible duration for reliable results
- Animals exhibit severe pain that cannot be alleviated must be euthanized immediately



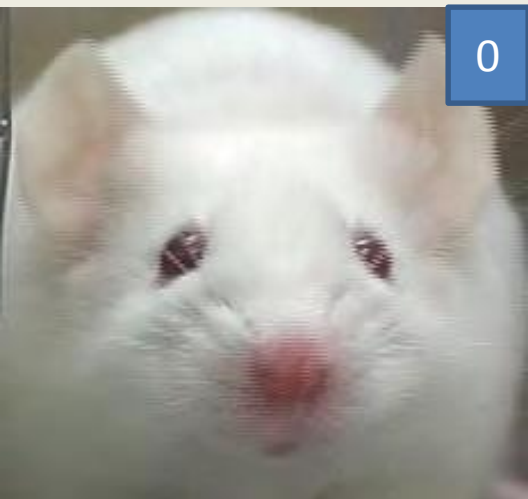
- While living, research animals should be provided the best living conditions
- This aspect is the direct responsibility of researcher/research team
- vocal expression of an animal in pain may not be in the audible frequency range of humans
- There is a special **pain scoring** system for every species of Laboratory Animals
- It usually demonstrates the parameters of normal behavior that changes in animals suffering pain

# Assessment of pain

- This pain scoring system must be used to guide researchers in determining whether analgesic therapy or euthanasia is indicated

**GRIMACE** scale is a useful tool to identify the intensity of pain in laboratory animals

- Orbital tightening
- Ear position
- Nose bulge
- Whisker change
- Cheek bulge

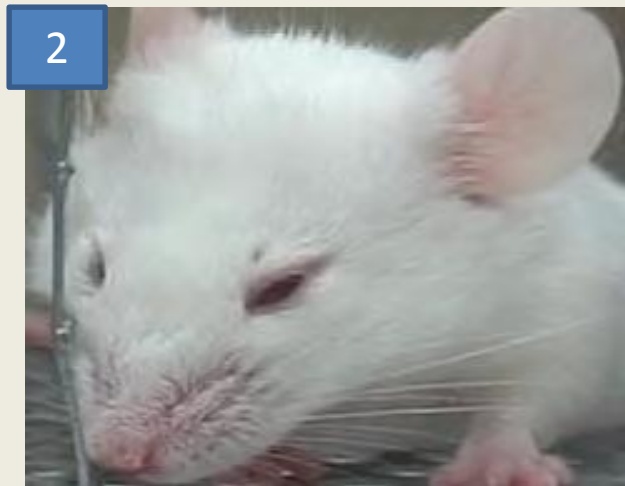


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OTRBITAL TIGHTNING



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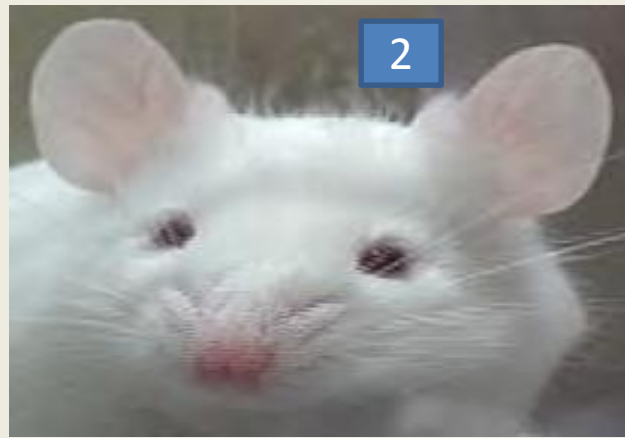


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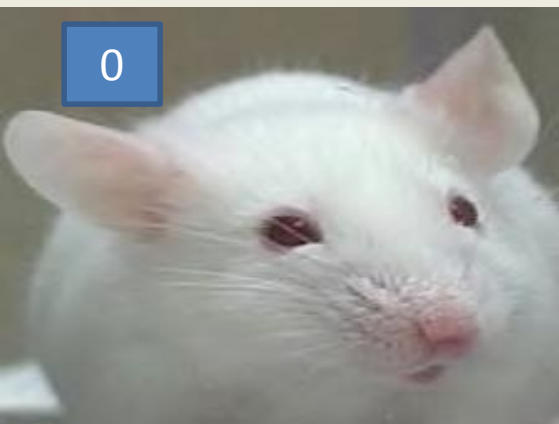


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NOSE BULGE



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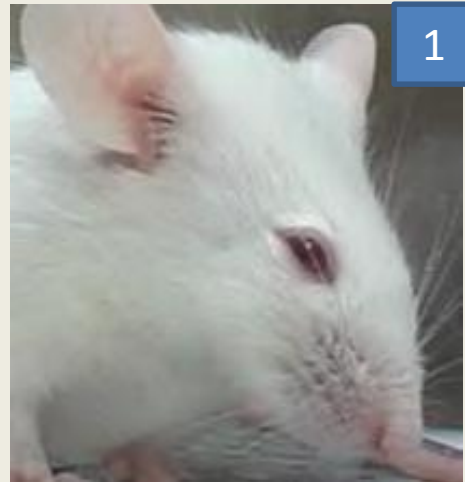
CHEEK BULGE



2



EAR  
POSITIONING



WHISKER  
CHANGE

# Rabbit GRIMACE Scale

Orbital Tightening		
		
Not Present [0]	Moderately Present [1]	Obviously Present [2]
The eyelid is partially or completely closed. The globes themselves may also be drawn in toward the head so that they protrude less, if the eye closure reduces the visibility of the eye by more than half, it would be scored as "2" or "obviously present".		

Cheek Flattening		
		
Not Present [0]	Moderately Present [1]	Obviously Present [2]
Contraction around the muzzle so that the whisker pads are pressed against the side of the face. The side contour of the face and nose is angular and the rounded appearance of the cheeks to either side of the nose is lost.		

Nose Shape		
		
Not Present [0]	Moderately Present [1]	Obviously Present [2]
The nares (nostril slits) are drawn vertically creating a more pointed nose that resembles a "V" more than a "U". The tip of the nose may also be tucked under towards the chin exaggerating this appearance.		

Whisker Position		
		
Not Present [0]	Moderately Present [1]	Obviously Present [2]
Whiskers are straightened and extended horizontally or pulled back toward the cheeks instead of the normal position where whiskers tend to have a gentle downward curve.		

Ear Position		
		
Not Present [0]	Moderately Present [1]	Obviously Present [2]
Normally the ears are roughly perpendicular to the head, facing forward or to the side, held in an upright position away from the back and sides of the body with a more open and loosely curled shape. In pain the ears rotate away from normal position to face towards the hindquarters, tend to move backward and be held closer to the back or sides of the body and have a more tightly folded or curled shape (i.e. more like a tube).		

# General pain scoring system

- **Activity** : activity levels generally decrease with pain.
- **Appearance**: Animals may be hunched or have a rough hair coat and have discharge around eyes and nose.  
( Porphyrin staining in rats may indicate pain or distress).

# Porphyrin staining in rats in pain.



# General pain scoring system continued.....

- **Temperament** :Animals may be aggressive
- **Vocalization** : Animals in pain make auditory noises (teeth grinding) when undisturbed in cage
- **Feeding behavior** : water and food intake is often decreased when an animal is in pain  
Reduction in body weight, hydration, urine or feces production may be measured



# General pain scoring system continued....

- **Physiological changes:**

respiratory rate and pattern, blood pressure, pulse, heart rate, skin colour or body temperature can be changed

- **Appearance in the surgery site :**

if animal has undergone a surgery for an experiment, erythema or swelling in the tissues around the incision is indicative of pain

# **Animal experiments can be done**

- 1. When alternatives are not available**
- 2. Expected outcome outweigh the possible discomfort of animal experiment**
- 3. People involved are trained ??**
- 4. Animal welfare is clearly maintained and supervised???**

# 3 R concept



- Three R concepts were introduced by British Scientists, Russell and Burch in 1959; defined by the term
- Reduction
- Refinement
- Replacement

# 1.Reduction

- Reducing the number of animals used in research by appropriate experimental designs
- Using suitable statistical applications
- Adequate literature review
- Work with internationally designated breeds of laboratory animals and internationally accepted protocols
- Prevent duplication of effort and minimize the number of animals

## 2. Refinement

Can be defined as the procedural refinement

- Using analgesia and anaesthesia in animal experiments
- Obtaining adequate training in animal handling/techniques
- Proper housing conditions
- Maintaining good management practices such as feeding/water
- Adequate space requirement and cage sanitation
- Avoiding unnecessary noises in animal rooms
- Environmental enrichment can be considered as procedural refinement

### 3 Replacement

- Total replacement of animal experiment with non animal methods
- It is the duty of all research scientists to look for alternatives
- Alternative methods may require advanced technology, skillful researchers and sufficient finance
- Research scientist should explore all the possible avenues to raise financial grants in order to develop alternative methods

# Animal Ethics Committees.



# What is an Animal Ethics Committee?

- As there are different ideas and different approaches concerning animal experimentations there should be universally accepted standards to follow
- According to accepted international standards there should be animal ethics committees in all research organizations where animal experiments are conducted
- This safeguards the research scientist as well as animal welfare requirement



# Who should be part of animal ethics committees?

- **Category A:** Veterinarian with a degree in veterinary science and experiences in working with **Laboratory Animals**
- **Category B:** Researcher or Teacher (usually with higher degrees) must demonstrate adequate experiences in recent research and teaching

- **Category C : Animal welfare person.**

- \*That person should have a demonstrable commitment and experiences in animal welfare

- \*Do not have any involvement with the institution

- \*Should not have any current involvement with care, supply or use of animals in research or training

- **Category D:** Independent person should not have current or past experiences in using animals for research or teaching purposes. Should bring independent thought and the **public perspectives** to the committee
- Other members: Animal care takers, who are involved with daily management requirements of animals

# Ethical Reasoning



# What is the importance of an 'Ethical Review' process?

## Provides a model for ethical reasoning:

- It is important to ensure good reasoning, justification and need for the experiment
- Ethical reasoning may help to assess the overall **scientific quality** and **validity** of the experiment
- Whether animals involved in the experiment exposed to suffering or adverse conditions
- Possibilities for reduction, refinement and replacement of the experiment
- **Adverse effect suffered by animals be compensated by the importance and outcome of the research**

# Why adequate training is essential before starting an animal experiment?

- Adequate training reduces the stress to the animals as well as to the researchers
- Improper handling can cause severe injuries to animals and researchers
- **Handling stress** can bring unwanted variables to the experiment which is minimized by proper handling

# What happen to the animals once the experiment is concluded?

## a) Rehabilitation:

- This is the process of re-homing the experimental animals as pets
- Rehabilitation steps should be clearly indicated in the research protocol when submitting the proposal for ethical approval
- Most international guidelines are advised to euthanize the animals once the experiment is completed.
- Releasing these animals to forests or desolate areas cannot be considered as a rehabilitation method

# What happen to the animals once the experiment is concluded?

- Laboratory animals are specifically designed for research purposes and releasing them to environment is considered harmful
- Animals which were used for non invasive and **non infectious experimental** protocols can be rehabilitated
- It is the responsibility of the research team or researcher to make a prudent judgment on this matter.

**Continued...**



# What happen to the animals once the experiment is concluded?

## b) Euthanasia:

- can be defined as gentle death. It is regarded as painless killing with minimum fear and distress.

**Continued.....**

# Why it is necessary to euthanize them?

- To collect various tissue samples for experimental processes
- Regulatory requirement when there is overcrowding
- Lack of adequate space requirement, funding to maintain animals after completion of the experiment
- Lack of adequate staff to maintain acceptable housing requirements

# Criteria for euthanasia.

- Euthanasia of animals is expected if animals demonstrate the conditions listed below.
- It is done in order to minimize pain and distress.
  - 1) Weight loss by 20%-25%
  - 2) Inappetence or complete anorexia for 24 hrs in small rodents
  - 3) Weakness or inability to obtain food or water
  - 4) Moribund stage - weak attempt to get up
  - 5) Infections - Not responding to antibiotics
  - 6) Signs of organ dysfunction

# Who is responsible for euthanasia?

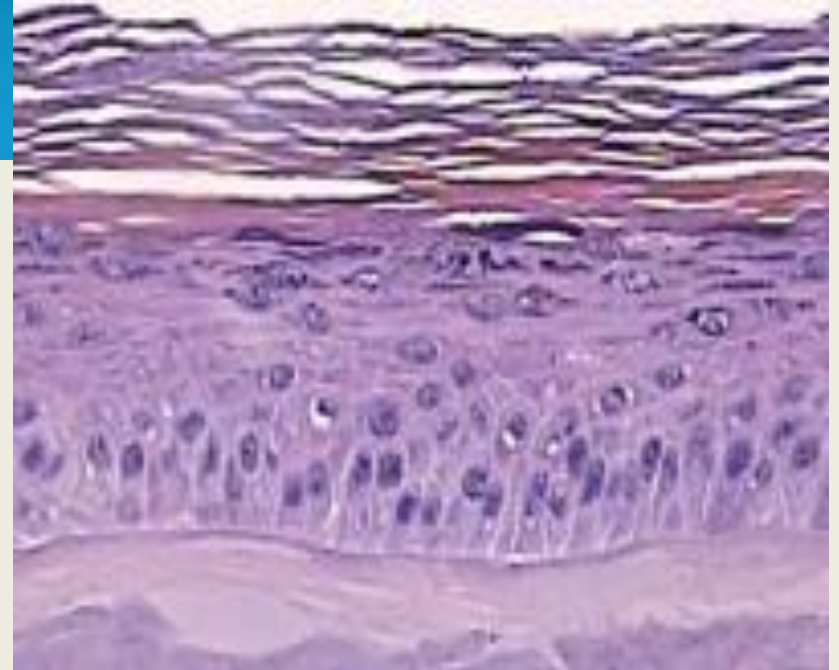
- Beneficiaries of the research should take the responsibility of euthanasia
- But line of duty should also be considered

- Research animals should have a comfortable daily existence, fulfilling their natural requirement
- They are totally dependent on the research scientists for their welfare, as they are in a confined laboratory environment
- As such, it is important to look at the situation from an animal's perspectives
- If animals are suffering as a result of experimental protocol and if there is a sign of severe pain which cannot be alleviated by using analgesia then euthanasia is recommended

- To achieve the welfare of research animals, ethical protocols, ethics committees and guidelines are essential
- Significant social value and impact of animal experiment could be achieved by careful selection and scrutiny of research proposal prior to approval

**WHEN IN DOUBT SUPPORT THE  
ANIMAL!!!**

# Alternatives to Animal Testing



# Alternative Methods in Animal Experiments.

- **Methods that enhance three R concept is defined as an alternative method.**
  - a) *In vitro*** : techniques such as tissue culture.
  - b) Lower species** : reduce the required number of vertebrate animals.  
E.g. bacteria, fungus, insects, molluskas.
  - a) Immunological techniques:** Their application is useful in diagnostic test, vaccine quality control, fundamental immunological research.



# Alternative Methods in Animal Experiments continued...

## d) **Physical –chemical method:**

e.g. High Pressure Liquid Chromatography (HPLC).

In the past, potency testing of products such as insulin, calcitonin, oxytocine required animal model. Now it has been replaced by HPLC.

## e) **Mathematical model and computer modeling**

e.g.: screening of series of related compounds.

# Alternative Methods in Animal Experiments continued...

f) **Human method:** Human or their tissues can justifiably be used as test subject.

e.g. human blood is used to screen pyrogens.

g) **Telemetry:**

Telemetry permits the continuous measurement of several parameters of freely moving animals.

E.g.: body temperature, blood pressure, heart rate, electro cardiogram etc by using miniaturized transmitters.

# Conclusion

- The use of Laboratory Animals for teaching and research is a fundamental necessity
- It is necessary for continuous progress of **Bio Medical Sciences**
- Use of animals constitute a special privilege to the scientific community
- It is incumbent upon each investigator and every member of their staff to fulfill all ethical and legal responsibilities
- It will enhance continues medical progress for the betterment of animals as well as for mankind

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