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# **Animal Experimentation**

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## **Animal Experimentation**

### **Preamble**

Animal research has been essential to advancing biomedical and veterinary science, providing crucial insights into biological processes, therapeutic strategies, vaccines, and treatments that form the foundation of modern medicine. This comprehensive module examines experimental animal use from historical, ethical, regulatory, technical, and biological perspectives, equipping students and future investigators across medicine, pharmacology, veterinary medicine, and life sciences with the knowledge necessary for responsible and optimal use of animal models in research. The course aims to develop critical understanding of the history and ethical evolution of animal experimentation, different types and applications of animal models, and the fundamental principles of the 3Rs (Reduce, Replace, Refine), while ensuring students can identify appropriate laboratory species, understand hygienic and legal regulations governing animal facilities, and master essential experimental techniques for biological data collection. Rather than celebrating animal use, this module places experimentation within a well-reasoned ethical and scientific framework, emphasizing the researcher's responsibility to minimize pain and distress, adherence to legislative requirements, technical precision in substance manipulation and response interpretation, and the development of critical thinking and ethical reasoning regarding scientific use of living organisms. Upon completion, students will be able to discuss experimental designs using animal models, explain the anatomy, physiology, and behavior of common laboratory animals, implement proper hygiene and ethical treatment protocols, and conduct or oversee experimental work in compliance with current legislation, building upon prerequisite knowledge of cellular and molecular biology, general physiology, animal classification, research ethics, laboratory biosafety, and proficiency in scientific English and French literature.

## **General Introduction**

Animal experimentation is an ancient and controversial practice involving live animals for scientific, medical, or educational research. This method has played a vital role in the development of biology and other scientific fields, allowing researchers to gain crucial insights into physiology, pharmacology, toxicology, and many other disciplines.

The different stories of Atation date back to ancient times, but it has developed significantly over the centuries, reaching its peak in the 20th century. Animal experiments have led to significant advances, such as the discovery of vaccines, the development of medical treatments, and the understanding of fundamental biological mechanisms.

However, animal experimentation also raises essential ethical and moral questions. It raises concerns about animal welfare, the suffering animals may endure during experiments, and the ethical justification for using them for human benefit. These concerns have led to intense debate and prompted governments and institutions to implement regulations aimed at regulating and limiting animal experimentation.

Furthermore, advances in scientific knowledge have also led to the development of alternative methods to animal testing, such as cell cultures, computer models and in vitro techniques, to reduce the need to use animals in research.

Today, animal testing remains a complex and controversial topic. Advocates highlight its benefits for science and medicine, while opponents emphasise the need to minimise animal suffering and promote alternative methods. This tension between scientific research and respect for animal welfare continues to spark debate and raise important symbolic questions around the world.

## **CHAPTER 1 HISTORY OF ANIMAL EXPERIMENTATION**

### **1. Introduction**

Animal testing has a long history dating back to ancient times, but has grown significantly in recent centuries. Here's a look at the history of animal testing:

**Antiquity:** The earliest references to the use of animals for experimental purposes date back to antiquity. The ancient Greeks, notably Aristotle, studied animals and performed dissections to better understand their anatomy. However, these experiments were primarily observations rather than experiments in the modern sense.

**Middle Ages:** During the Middle Ages, animal experimentation was limited due to religious and philosophical beliefs that placed great value on animals. However, some advances were made in veterinary medicine, particularly regarding the care of farm animals.

**Renaissance:** The Renaissance period marked a return to interest in animal experimentation. Anatomists such as Andreas Vesalius used animals to better understand human anatomy, contributing to medical advances.

**17th and 18th centuries:** Animal experimentation developed during this period, primarily in medicine. Scientists conducted experiments on animals to study physiological functions and to develop vaccines and treatments.

**19th century:** Vivisection (dissection of live animals for experimental purposes) became more common in the 19th century. Advances in physiology and medicine have been partly attributed to these experiments, but they also raised ethical concerns.

**20th century:** In the 20th century, animal experimentation expanded significantly. It was used in many fields, including medical research, toxicology, drug research, biology, and psychology. However, it also sparked debates and animal rights movements.

**Ethics and Regulation:** Over time, the issue of the ethics of animal testing has grown in importance. Regulations have been implemented in many countries to regulate and limit the use of animals for experimental purposes. Alternatives to animal testing, such as cell cultures and computer models, have also been developed to reduce the need for animal testing.

Today, animal testing remains controversial, with ethical concerns and debates over the necessity and justification of its use in scientific research. Regulations vary from country to country, but

they generally aim to minimise animal suffering and encourage the development of alternative methods.

## **2. Definition of animal testing**

Animal experimentation, also known as animal research, refers to the practice of conducting experiments or tests on living animals to obtain scientific, medical, pharmaceutical, or other types of knowledge. These experiments may include observations, manipulations, surgical procedures, tissue sampling, drug testing, behavioural studies, and other activities better to understand logical, pharmacological, or behavioural processes.

Areas of application of animal experimentation: According to statistical surveys, there are eight major areas of application of animal experimentation: basic research, biomedical research, toxicology, pharmacology, cosmetics, agri-food, environment and safety. Basic research aims to develop knowledge and understanding of phenomena without direct application, while biomedical research concerns drugs, vaccines, treatments, chemicals, plant protection products, etc.

## **3. Advantages and disadvantages of animal testing**

### **3.1 Benefits**

Scientific and medical advances: animal experimentation has contributed significantly to advancing our knowledge of living things, improving the health and lives of humans, and understanding Diseases and Biological Processes. Animals are used as models to study disease and to understand biological processes.

Product safety for humans: New drugs must be tested to measure a compound's beneficial and harmful effects on the entire population.

Development of treatments and drugs: Animal testing is necessary to develop and test new therapies and medicine.

### **3.2 Disadvantages**

**Animal suffering:** Animal testing often involves painful and stressful procedures for the animals.

**High cost and ineffectiveness:** Experiments on non-human animals can prolong human suffering associated with effective treatments.

**Reliability of results:** Test results from other species often cannot be reliably extrapolated to humans.

**Ethics:** The use of animals for research raises ethical and moral questions.

#### **4. The most commonly used animals in animal testing**

There are many animal species models for animal experimentation. The most widely studied are mice, rats, and rabbits, but others, including guinea pigs, monkeys, and monkey experimentation, speak more of an animal model.

An animal model allows us to study spontaneous or induced biological, physiological or even pathological phenomena that have one or more aspects in common with humans.

The animals most commonly used in animal testing vary by country and year, but mice, rats, rabbits, fish, and birds are the most widely used species.

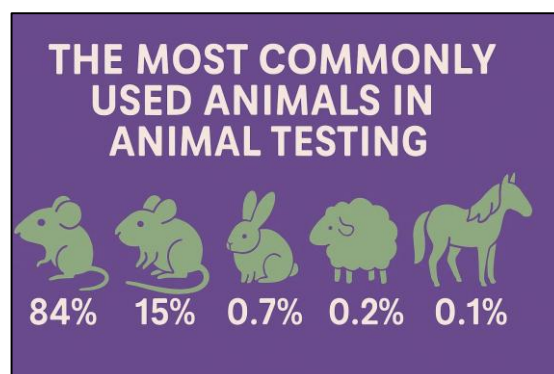
In France, mice represent 62% of the animals used, followed by fish (22%), rats (8%), rabbits (7%), chickens (2%) and guinea pigs (2%).

In Switzerland, more than 60% of laboratory animals are mice, followed by rats, birds and fish.

Animals used for animal testing include cats, dogs, monkeys, and other species.

According to the Algerian Pasteur Institute's Annual Report 2015, 27% of Algerian institutions used animals in research and/or teaching, with 37,958 used yearly. The University of Sétif, the Houari Boumediene University of Science and Technology of Algiers (USTHB), and the University of Oran used the most significant number of animals, 13%, 11% and 9.5%, respectively, of the total number of animals used by the twenty-seven institutions.

The frequency of species used was 84%, 15%, 0.7%, 0.2% and 0.1%, respectively, for mice, rats, rabbits, sheep and horses.



**Figure 1:** The most commonly used animals in animal testing

## 5. The different animal models

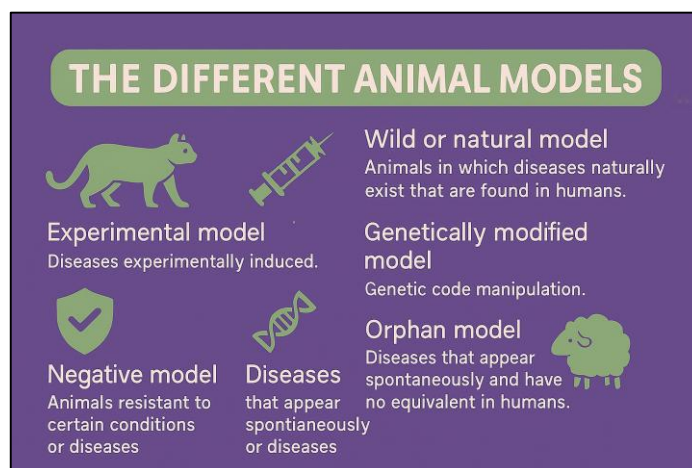
**Wild or natural model:** This model consists of animals that have not undergone any modification or manipulation; rather, it is an animal in which diseases naturally exist that are found in humans, such as diabetes, high blood pressure, arthritis, etc.

**Experimental model:** it is a model in which pathology is experimentally induced, such as cancer or even diabetes mellitus induced by the injection of streptozotocin. This chemical molecule causes destroys cells.

**Genetically modified model:** This is a model whose genetic code has been modified. The purpose of manipulating the genetic code is to induce a disease that exists in humans to better study it in animals, identify its causes and propose possible treatments. These manipulations consist of inserting a fragment of DNA, replacing it, or even neutralising it, which results in genetically modified strains such as "knock-out" strains.

**Negative model:** This model consists of animals resistant to certain conditions or diseases, which is why the search for the reasons or factors of resistance is of great interest to human health.

**Orphan model :** This model encompasses animals with diseases that appear spontaneously and have no equivalent in humans, such as scrapie in sheep.



**Figure 2:.** The different animal models

### 5.1 Criteria for selecting animals for animal experimentation

Criteria for selecting animals for animal testing may vary depending on the study in question, but here are some commonly used criteria:

**Availability:** Animals must be available in sufficient quantity for the survey and they must be affordable for researchers

**Size:** Animals should be of appropriate size for the study in question

**Lifespan:** Animals must have a sufficient lifespan to allow the study.

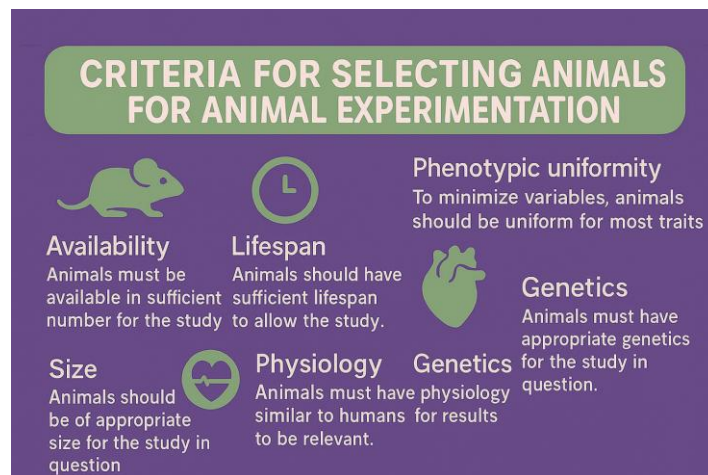
**Physiology:** Animals must have physiology similar to humans for results to be relevant.

**Genetics:** Animals must have appropriate genetics for the study in question.

**Phenotypic uniformity:** To minimise variables, animals should be uniform for most traits, such as coat colour.

**Relevance to the study:** The animals must be relevant to the study in question; for example, dogs are used to study heart disease because their physiology is similar to that of humans.

It is important to note that alternative methods to animal testing are also used to reduce the number of animals used and, in some cases, completely replace the use of animals.



**Figure 3:** Criteria for selecting animals for animal experimentation

## CHAPTER 2: REGULATORY AND ETHICAL ASPECTS OF ANIMAL EXPERIMENTATION

### 1. Introduction

European and French texts govern animal research at the legislative and regulatory levels, illustrating the growing consideration of animal welfare.

With the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (), developed by the Council of Europe in 1985, member states affirmed their moral duty to respect all animals used for scientific purposes. They committed to reducing the number of experiments and animals used in research, encouraging the development of alternative methods and using animal models only when no other relevant methods are available to meet the purpose of a study.

From this convention stems the European Directive on the protection of animals used for scientific purposes, adopted in 1986 by the Council on the approximation of the laws, regulations and administrative provisions of the Member States relating to the protection of animals used for experimental and other scientific purposes (Directive 86/609/EEC). This Convention was revised in 2010 by the European Parliament and of the Council on 22 September 2010 on the protectingly used for scientific purposes (**Directive 2010/63**).

Generally speaking, this regulation is based on the 3 R rule (Replacement, Reduction and Refinement), which consists of Replacing animals as much as possible, Reducing their number in studies and improving their conditions of use.

### 2. Rule of 3 Rs

Developed in 1959, the 3Rs rule forms the basis of the ethical approach to applied experimentation in Europe and North America. Its requirements accompany any research project that uses animals. The 3Rs principle was born from the ethical reflections of two English biologists, William Russell and Rex Burch. In their 1959 book, "The Principles of Humane Experimental Technique," the two scientists first set out this principle to reduce, replace, and refine animal experiments.

This principle was gradually adopted by various institutions and introduced into regulations, particularly in Europe and France. It contributes to the development of alternative methods for animal testing.

### **2.1 Reduce**

The limitation to only those experiences considered essential reducing unnecessary repetition of previous studies the writing of an experimental protocol before any experimentation, knowing that a well-prepared study often makes further tests on animals unnecessary

While it is essential to use the smallest possible number of animals, care must be taken to avoid this reduction effort harming the reliability of the results. Biostatistical considerations must, therefore, be incorporated into the project design.

### **2.2 Replace**

It is sometimes possible to work on cells or tissues (in vitro) or even on digital models (in silico) to replace animal models. If the objective of the researcher's experiment allows it, the use of in vitro or in silico models should be the objective. Although this objective is achievable in many fields, particularly in safety studies in the broad sense, alternative methods remain complementary to animal models.

Looking for alternative methodDevelopingof methods to replace animal models is a field of research in its own right. Thus, the Cell Institute for the Treatment and Study of Monogenic Diseasesd 'Évry currently works on stem cells.

However, the extraordinary complexity of living things challenges certain in vitro or in silico models. "Disease modeling is still very imperfect," explains Bruno Villoutreix, the unit's director. Similarly, it is not yet possible to predict the side effects of biotherapies—monoclonal antibodies, gene and cell therapies—because data are lacking, and understanding of some mechanisms is still limited. On the other hand, predicting some side effects is possible for small chemical molecule drug candidates.

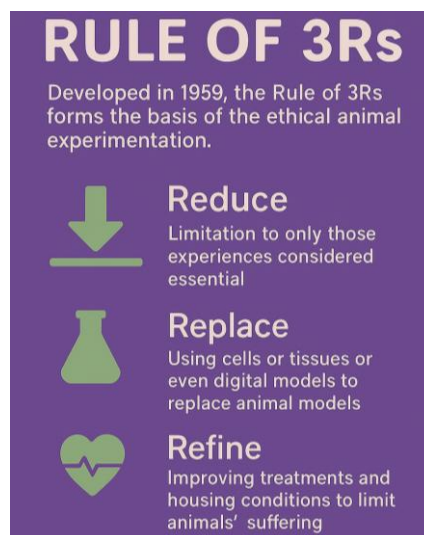
Although in vitro and in silico methods remain complementary today and cannot replace all studies carried out on animals, they contribute to them in part.

### **2.3 Refine**

"Refining" means optimising animal experimentation by reducing, eliminating, or alleviating their pain and distress to improve their well-being. Before the experiment, this includes carefully choosing the appropriate animal model, improving transport, breeding, and housing conditions, planning protocols that minimise stress, training animals to cooperate in non-invasive and painless procedures, and setting clear endpoints to avoid unnecessary suffering.

During the experiment, refinement focuses on the procedures used. Priority should be given to non-invasive methods such as imaging or telemetry, ensuring proper care before, during, and after operations, using adequate anaesthesia and analgesia, reducing the duration of studies when possible, especially in toxicology, and applying humane euthanasia practices when needed.

After the experiment, refinement involves making the best use of the results obtained while recognising that scientific data is only valid if the animals were properly cared for. In this way, ethical responsibility aligns with scientific quality, after all, "happy animals make good science."



**Figure 4:** Rule of 3 Rs

### **3. What is a “breakpoint”?**

The endpoint is when an animal's suffering and/or distress is stopped or minimised. Therefore, each experimenter must know how to identify physical and emotional suffering, quantify it using a scale, and act when predefined termination criteria are reached.

#### **3.1 The main principles of regulation**

Animal research is only lawful if it is "strictly necessary." Researchers are "determined to limit the use of animals for experimental purposes to replace such use wherever possible." This point is stated in Convention ETS 123.

#### **3.2 Authorisation of projects**

To begin with, any research project using animals must have received authorisation from the Ministry of Research. Authorisation is issued for a maximum period of 5 years, provided that the project has received a favourable evaluation from the institution's ethics committee where it will be carried out.

#### **3.3 A compliant and approved establishment**

Any establishment that uses animals for scientific purposes must hold an accreditation. This is issued by prefectural decree after visits by veterinary inspectors from the Departmental Directorate for Social Cohesion and Population Protection (DDPP or DDCSPP). Valid for 6 years, this accreditation depends on the Ministry of Agriculture and must be renewed upon written request. Its issuance is based on compliance with housing and operating standards set by ministerial decree to ensure animal welfare. The same constraints apply to establishments breeding or supplying animals.

The use of genetically modified animals requires additional approval, which is issued by the Ministry of Research.

#### **3.4 Competent and trained staff**

All personnel handling animals must possess appropriate qualifications and ensure their skills are maintained and updated. In addition to specific initial training, continuing education of at least three days every six years is now required. Personnel who work with non-domestic species must also hold a certificate of competence for the care and breeding of the species in question.

A reasoned choice of species associated with a minimum number of animals

The European directive specifies that projects "use the minimum number of animals to obtain reliable results and require the use, among the species least likely to experience pain, suffering, distress or sustained harm, of those that are optimal for extrapolation to the target species."

#### **An identified origin of animals**

"Animals used or intended to be used in experimental procedures must have been bred for that purpose and come from approved breeders or suppliers."

#### **A reduction in constraints**

The constraints imposed on animals during experiments must be reduced to a minimum. This principle has been affirmed since Convention ETS 123: "When choosing between procedures, those which cause the least lasting damage, pain, suffering and distress and which are likely to give the most satisfactory results should be selected."

### **4. The ethical system**

#### **4.1 A charter**

Since 1992, a charter on the ethics of animal experimentation has been developed in accordance with the 10 measures set out by the Minister of Research, Hubert Curien. These measures aimed to develop a genuine ethical policy for animal experimentation in public research organisations. This charter has since been revised and adapted to changes in regulations.

#### **Article 1: Respect for animals**

The ethics of animal experimentation are based on the duty of humans to respect animals as living and sensitive beings capable of feeling pain, suffering and anguish.

#### **Article 2: Individual responsibility**

Using animals for experimentation engages the moral responsibility of each person involved.

#### **Article 3: Responsibility of institutions**

Institutions are morally responsible for experiments carried out within them or on their behalf of animals.

#### **Article 4: Skills**

Responsibility requires ethical training and regulatory, scientific, and technical skills appropriate to the species used, which are duly updated at all levels of intervention. These skills are sought as

often as necessary from specialists in the physiology, ethology, or medicine of the animals concerned.

**Article 5: General principles**

A reflection on the scientific, ethical, and societal merits of using animals must precede any experimental approach. Methods and techniques to eliminate or reduce to a strict minimum harm to animals must be systematically sought. The development and promotion of these methods and techniques must be widely encouraged.

**Article 6: The ethical approach to animal experimentation**

- Any experimentation involving animals is preceded by consideration of the following:
- The absence of adequate alternative methods to pursue an identical purpose,
- The usefulness of the experiment envisaged about work conducted elsewhere,
- The relevance of the methods chosen and the level of probability of achieving tangible results,
- The adequacy between the animal models considered and the scientific objectives pursued,
- The significance of animal harm in related results,
- Taking into account the biological and cognitive characteristics of the species concerned,
- The choice of species, when it comes to non-domestic species so that it does not threaten biodiversity,
- Limiting the number of animals used to the minimum necessary,
- The choice of living conditions, accommodation, care and use of animals so that their physiological and behavioural needs are respected as much as possible.

**Article 7: Role of ethics committees in animal experimentation**

- Each ethics committee must constitute a body for dialogue and reflection.
- It provides advice on projects using animals for scientific purposes, referencing the principles set out in this Charter.
- These opinions are justified and may be accompanied by recommendations.
- Each ethics committee must participate in promoting the ethical principles set out in this Charter.

**Article 8: Composition of ethics committees for animal experimentation**

- Each ethics committee brings together multidisciplinary expertise and informed opinions. Civil society and veterinary medicine are represented.

**Article 9: Ethics of ethics committees in animal experimentation**

- Every ethics committee is independent and impartial and guarantees the confidentiality of the submitted files.
- It considers the opinions or recommendations of the National Committee for Ethical Reflection on Animal Experimentation.

## **5. Ethics committees in animal experimentation**

The application of the directive led, in the 1990s, to the creation of ethics committees responsible for assessing the acceptability of protocols regarding the relevance of the study and the harm caused to animal welfare. Initially optional, the ethical assessment of projects by ethics committees in animal experimentation became mandatory in 2013.

### **5.1 Ethics committees for animal experimentation**

Animal Experimentation Ethics Committees (CEEA) are recognised as the competent authority for the ethical evaluation of applications for authorising projects using animal models. CEEAs comprise at least five people: a veterinarian, a researcher, an experimenter, an animal handler, and a member of the public not involved in research activities.

## **CHAPTER 3: HYGIENE AND HEALTH CONTROL OF A PET SHOP**

### **1. Introduction**

Hygiene and health control in a pet store are paramount to ensure the health, welfare, and safety of the animals housed there and prevent the spread of disease. A pet store is where various animal species are kept, sold, or displayed and is, therefore, an environment conducive to transmitting pathogens. A rigorous approach to hygiene and health control is necessary to ensure optimal conditions for the animals and to meet the expectations of customers concerned about the well-being of their future companions.

First and foremost, hygiene involves implementing cleaning, disinfection, and waste management protocols to maintain a clean and healthy environment. This includes the cleanliness of cages, tanks, storage areas, and workspaces, as well as the proper management of bedding, uneaten food, and waste. Preventing contamination is essential to minimise the risk of communicable diseases. Health control, on the other hand, involves closely monitoring animal health, isolating those showing signs of illness, and implementing procedures to prevent the spread of disease. Sick animals must be isolated and treated by a qualified veterinarian. In addition, it is important to implement programs to prevent and control parasites, which are a common threat to animal health in pet stores.

Animal facility staff are key in implementing these hygiene and health control practices. They must be trained in good practices, particularly regarding personal hygiene, animal handling, and recognising signs of disease. Animal care, including feeding, watering, and managing contact with the public, must be carried out with the utmost care.

Additionally, it is essential to comply with local and national regulations regarding the health control of pet stores. Regular inspections by a veterinarian are often required to ensure that animals receive proper care and that the pet store complies with public health standards.

**The main points to consider are**

- **Cleaning and disinfection**

Pet store premises, including cages, storage areas, and workspaces, must be cleaned and disinfected regularly. Use appropriate disinfectants and follow the manufacturer's instructions.

- **Waste management**

Waste, including bedding, uneaten food and faeces, must be disposed of properly and regularly to prevent odours, pest control and contamination.

- **Isolation of sick animals**

Any animal showing signs of illness should be isolated from other animals to prevent the spread of the disease. A veterinarian should be consulted to evaluate and treat sick animals.

## **2. Planning for daily care**

A schedule for daily animal care should be established

- **Pest control**

Implement a pest prevention and control program to prevent infestations. This may include the use of appropriate pest control treatments.

- **Personal hygiene**

Staff must follow strict hygiene practices, including frequent hand washing, wearing clean clothing, and limiting contact with sick animals.

- **Feeding and watering**

Animals must be provided with adequate food and water. Food must be stored in clean containers, and bowls must be cleaned regularly.

- **Temperature and humidity control**

Ensure that environmental conditions, such as temperature and humidity, are appropriate for the specific needs of the animals you house.

- **Staff training**

Animal facility staff must be trained in good hygiene and animal care practices. This includes recognising signs of illness and handling animals properly.

- **Animal health registration**

Keep accurate records of each animal's health, including vaccinations, medical treatments, and veterinary visits.

### **Regular inspections**

A veterinarian should conduct regular inspections to assess animal health and compliance with health standards.

- **Limiting the number of people**

For animal safety reasons, a limited number of people must be allowed inside the pet store.

- **Health emergency plan**

Develop an emergency plan for an epidemic or contagious disease and ensure staff know how to implement it.



**Figure 5:** Planning for daily care

## **3. Organization, Equipment and Materials of a Pet Shop**

### **3.1 Organization of a pet store**

The animal house which is supposed to house the laboratory animals must be designed according to international standards, the layout of the premises is as follows:

- Premises intended for animals (accommodation, quarantine, isolation)
- Laboratories and experimental rooms
- Food and Litter Storage
- Receiving area (facilitate access to the animal facility for vehicles for receiving and shipping)

### **3.2 Cleaning/laundry facilities**

- **Circulation areas:** Circulation areas must be designed to meet the housing standards for animals present in the establishment and allow for the circulation of rolling stock. They must also reduce contamination between animal housing rooms and limit staff's unnecessary exposure to animals and their waste. Circulation is organized around the cleaning room and housing rooms.
- **Staff Rooms:** Changing rooms should allow staff to change their clothing and shoes. They should be located between the entrance hall and the animal facility. Their design varies depending on the animal facility's status. Offices and the break and meeting room should preferably be located next to the animal facility.
- **Technical facilities:** To facilitate the work of maintenance teams, it is best to group all technical installations in a single room outside the animal facility. This way, technical teams do not need to enter the animal facility.

The design of the pet store must meet the animal's needs in terms of comfort and well-being.

The pet shop must be built in places away from public areas.

- **The pet store must be designed to facilitate cleaning and disinfection. To do this:**

To prevent the development of certain parasites, fungi, and others, the walls, ceilings and surfaces must be made of durable, waterproof materials that are easy to clean and disinfect.

The pet store must contain several rooms or boxes where the animals are kept separately according to species to avoid contamination and the stress created by certain species.

Monitor the pet store's environmental conditions while respecting each species' requirements.

### **3.3 Compliance with environmental parameters:**

- **Ventilation:** The purpose of the ventilation system is to bring clean air into the accommodation space, to reduce odours, toxic gases, dust, potential infectious agents, excessive heat and humidity and, if necessary, to create air pressure gradients between adjoining spaces.
- **Ambient temperature:** Ambient temperature is a parameter that directly impacts relative humidity and animal metabolism, and it is approximately 20-24°C.
- **Lighting:** The frequency, intensity, and wavelength of light impact the reproduction, behaviour, and physiology of mammals. Light is also important for synchronising the circadian rhythm. Lighting is provided for 12 hours per day.

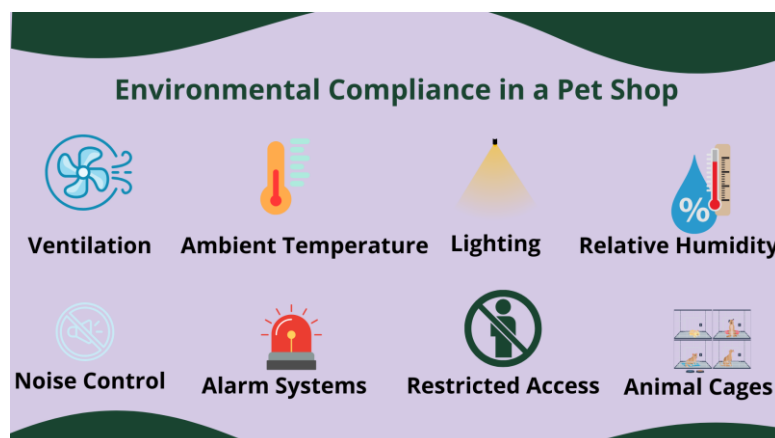
- **Relative humidity:** Raising relative humidity directly impacts animals' thermoregulatory capacity, as evaporative heat loss is essential in maintaining homeostasis. The tolerated humidity is 40-60%, and relative humidity should be maintained at  $45\% \pm 10\%$ .
- **Noise:** The pet store must have a soundproofing system to reduce stress caused by certain species, such as barking dogs.
- **Alarm system:** It is recommended that systems be provided to detect fires and the entry of unauthorised persons. Since technical faults or breakdowns in ventilation and heating systems can cause disturbances or even death to animals, it is also recommended that monitoring devices be installed to allow staff to monitor the operation of these two systems. Finally, if necessary, it is recommended to provide a backup generator to ensure the operation of the devices, ensuring the animals' survival and lighting during power outages. Alarm systems must disturb the animals as little as possible.

Access to the animal facility must be reserved only for authorised personnel to limit the risks of contamination from animals to humans or vice versa.

Staff must protect themselves by wearing a coat, a bib, gloves, overboots, etc.

- **Animal cages:** The cages in which the animals are kept must meet the requirements of each species. The cages must be made of stainless steel, easy to clean and disinfect, and equipped with a bottle or teat for watering, a hopper for food, and nest boxes for the young, in the case of rabbits.

The litter, generally sawdust, must be renewed periodically to prevent the development of parasitic agents.



**Figure 6:** Environmental Compliance in a Pet Shop

#### **4. Pet Shop Equipment: Description, Use and Maintenance**

The equipment and materials used in a pet store vary depending on the specific needs of the animals housed there. Here is a list of commonly used equipment, along with their description, use, and maintenance tips:

##### **4.1 Fixed equipment**

- **Airlock:** Entrance equipment may consist of a double-door staff airlock with a code system to control entry into the animal facility, rodent barriers and other protective measures to combat plague.
- **Sterilisation equipment:** This equipment can consist of a double-entry autoclave, a bactericidal bath, an ultraviolet airlock, and a formalin airlock.
- **Washing equipment:** Manual washing using cleaning and disinfecting products is possible in certain cases, such as in small pet shops, but is not recommended.
- **Automatic watering systems:**
- **Water filtration equipment**

*Description:* Filters for purifying water in aquariums and terrariums.

*Use:* Maintain water quality suitable for fish and other aquatic animals.

*Interview:* Filters should be cleaned and maintained according to the manufacturer's recommendations.

- **Heating and lighting**

*Description:* Heating and lighting systems to maintain appropriate environmental conditions.

*Use:* Ensure adequate temperature and lighting for animals.

*Interview:* Heating and lighting systems should be checked regularly to ensure they work properly.

- **Incubators and heat lamps**

*Description:* Equipment for incubating eggs and maintaining warmth for newborns.

*Use:* Incubate animal eggs and maintain appropriate temperatures for young animals.

*Interview:* Clean incubators regularly and check the temperature of heat lamps to avoid overheating.

##### **4.2 Mobile equipment**

- **Cages and enclosures**

*Description:* Cages and enclosures are temporary housing for animals.

*Use:* They house animals, isolate them if necessary and present them to the public.

*Interview:* Cages should be cleaned regularly, bedding should be changed, and covering materials, such as newspapers, should be replaced. Cage surfaces should be disinfected periodically.

- **Bowls and waterers**

*Description:* Bowls for food and drinking troughs for water.

*Use:* Provide food and water to animals.

*Interview:* Bowls and waterers should be cleaned daily to prevent contamination. Wash them with a mild detergent and warm water, then rinse them thoroughly.

- **Quarantine cages**

*Description:* Cages specially designed to isolate sick or newly arrived animals.

*Use:* Separate sick or recently arrived animals from others to prevent the spread of disease.

*Interview:* Quarantine cages must be cleaned and disinfected after each use.

- **Reptile cages and terrariums**

*Description:* Housing specially designed for reptiles.

*Use:* House reptiles, including snakes, lizards and turtles.

*Interview:* Reptile cages and terrariums should be cleaned regularly, and substrates, such as sand or bedding, should be replaced.

- **Transport cages**

*Description:* Cages or boxes for transporting animals.

*Use:* Moving animals safely.

*Interview:* Clean cages after each use to prevent disease transmission.

- **Grooming products:** Grooming products clean and maintain animals. They should be chosen according to each animal's specific needs and used according to the manufacturer's instructions.
- **Food:** Pet food must be stored properly to avoid contamination and spoilage. Expiration dates should be checked regularly, and expired food should be discarded.
- **Measuring instruments:** Measuring instruments measure food, medications, and other products for animals. They must be cleaned regularly to avoid cross-contamination.

Inventory management software tracks stock levels and orders and must be updated regularly to reflect current stock levels.

Regular maintenance, cleaning, and disinfection are essential to ensure animals' health and well-being and pet store staff's safety. Always follow the manufacturer's recommendations for maintaining each piece of equipment and ensure staff are trained in these procedures.



**Figure 7:** Pet Shop Equipment

## **5. Prevention of risks related to laboratory handling**

Preventing risks associated with laboratory manipulation is essential to ensure staff safety, the quality of research results, and environmental protection. Here are some key steps to minimize risks in a laboratory:

- **Staff training**

Ensure all laboratory staff are trained in safety protocols, handling specific equipment, and chemical management. This also includes training on how to respond during an incident.

- **Risk assessment**

Identify potential risks associated with laboratory procedures and substances. Create a list of hazardous chemicals, reagents, and high-risk equipment, and assess the associated hazards.

- **Personal Protective Equipment (PPE)**

All staff must wear appropriate personal protective equipment (PPE), such as gowns, gloves, safety glasses, and closed-toe shoes. PPE must also be of good quality and properly maintained.

- **Handling and storage of chemicals**

Strictly follow chemical handling protocols. Ensure reagents are properly labelled and stored in specific areas and that safety data sheets are available for each product.

- **Ventilation and emission control**

Ensure adequate ventilation in the laboratory to eliminate potentially harmful fumes and emissions. Install fume hoods when working with volatile chemicals.

- **Safety equipment**

Regularly check the condition of safety equipment, such as eyewash stations, fire extinguishers, and fire detection systems, to ensure they are in good working order.

- **Waste management**

Properly dispose of chemical and biological waste. Establish procedures for collecting, temporarily storing, and safely disposing of hazardous waste.

- **Emergency measures**

Develop emergency plans and ensure staff know how to respond to incidents such as chemical leaks, fires, or exposure to hazardous substances.

- **Compliance Monitoring**

Implement a system for monitoring and verifying compliance with security protocols. Conduct regular audits and inspections to ensure standards are being met.

- **Communication and awareness**

Regularly inform staff of potential risks and safety measures. Encourage open communication regarding safety concerns.



**Figure 8:** Prevention of risks related to laboratory handling

## 6. Documentation and recording of incidents

All laboratory incidents and accidents must be investigated and recorded to prevent repeat occurrences.

Risk reduction is based on vigilance, training and respect for the safety procedures. Continual dedication is necessary for a safe and positive environment.

### 6.1 Animal Welfare

These effects on the biological and physiological conditions of animals may differ under these conditions of well-being and result in biotechnological research involving some issues that have been erroneously identified.

These are environmental factors (temperature, air, humidity, light, and noise) and hazardous infection agents for animal health.

## **6.2 Infectious Agents**

Several pathogens (bacteria, fungus, mites, protozoa) may induce diseases that affect the research outcome.

These diseases can be passed between different animals or between animals and humans.

Contact: skin or STDs.

- **Indirect transmission:** air, water, soil, or fomite (e.g., respiratory PAS).
- **Passive vectors:** fomites (cages, food, syringes, scissors, etc.).

## **6.3 Recommendations**

- Take rigorous precautions.
- Disinfect or cull infected animals.
- Access to the animal facility should be restricted.
- Quarantine new arrivals until health is confirmed.

## **7. The pain**

Pain is the most unpleasant sensation that an animal can feel during the experiment. It can be physical pain caused by the various manipulations or stress and anxiety caused by separation, the type of detention or other factors.

Since animals are living beings with a nervous system, they feel pain just like humans, except that the expression of pain is different. Therefore, monitoring the animal's behaviour seems to be the best way to recognise its well-being and health, requiring qualified personnel.

Pain is the most unpleasant sensory and/or emotional experience that an animal can feel. It is caused by tissue damage that provokes motor reactions expressed by flight, fear, and isolation.

### **7.1 Signs of pain**

The expression of pain varies from one animal species to another, from one individual to another, and even from one experience to another; however, there are signs that are common to all species.

Common signs

- Physiological signs include:
  - Tachycardia (rapid heart rate)
  - Increased breathing rate

Modification of neuroendocrine parameters following the secretion of certain stress hormones, including adrenaline, noradrenaline, and cortisol.

Behavioral signs include a reduced appetite and decreased exploratory behavior, as well as attempts to escape or defend themselves during handling. Animals may also emit vocalizations such as groaning, moaning, or howling. In severe cases, self-mutilation may occur, including limb autophagy. Apparent signs include a spiky, disheveled, dull, and poorly maintained coat, changes in facial expression, particularly in the eyes, and unusual posture. Specific signs may vary depending on the species.

## **7.2 The pain levels**

Different manipulations and practices cause four increasing levels of pain.

### **7.2.1 Level 0 degree of stress 0**

Manipulations that do not cause the animal pain or anxiety and do not require authorization from ethics committees to carry out the protocol.

Examples include blood sampling for diagnostic purposes, injection of non-irritant products under the skin, and observation of behaviour.

### **7.2.2 Level 1 light constraint of degree 1**

Manipulations causing short-term light constraints

Example: skin biopsy, injection of mildly irritating products, castration of males under anesthesia

### **7.2.3 Level 2 average stress of degree 2**

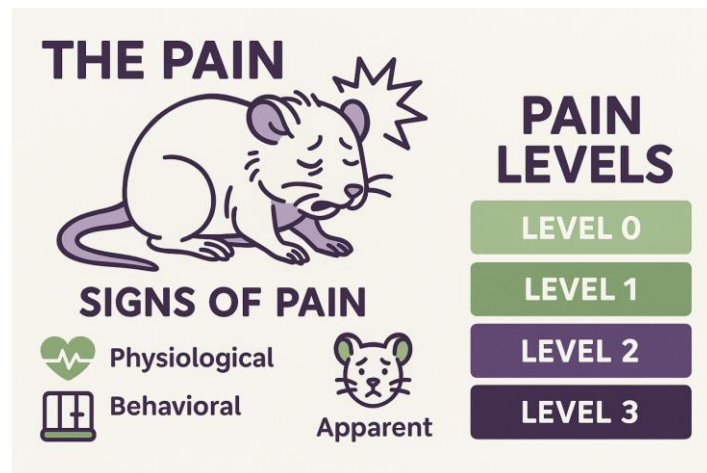
Manipulations causing medium or moderate stress of short duration or light stress of medium to prolonged duration.

Example: exploratory laparotomy and injection of highly irritating products.

### **7.2.4 Level 3 severe constraint of degree 3**

Manipulations causing severe or light constraints of prolonged duration

Example: deep tissue damage, infectious disease, induced cancers leading to death without euthanasia.



**Figure 9:** Indicators and Severity Scales for Pain in Animal Models

### 7.3 Treatments

In animal experimentation, assessing and managing pain and distress is a critical aspect of ethical research and scientific validity. After defining and estimating the degree of constraint and pain caused by various manipulations, researchers must implement interventions that are either preventive or curative to relieve animal suffering. Pain and stress, if left unaddressed, not only compromise animal welfare but can also affect physiological and behavioral responses, ultimately impacting the reliability and reproducibility of research results.

Interventions are tailored according to the severity of the pain or distress, which is generally categorized into graded levels. At level 0, animals experience minimal or no discomfort, and no specific analgesic or anesthetic treatment is required. For level 1 constraints, which involve mild pain or minor manipulations, treatment typically includes analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) and, if necessary, low-dose morphine. These interventions help control pain while maintaining normal behavior and physiological parameters, ensuring minimal interference with experimental outcomes.

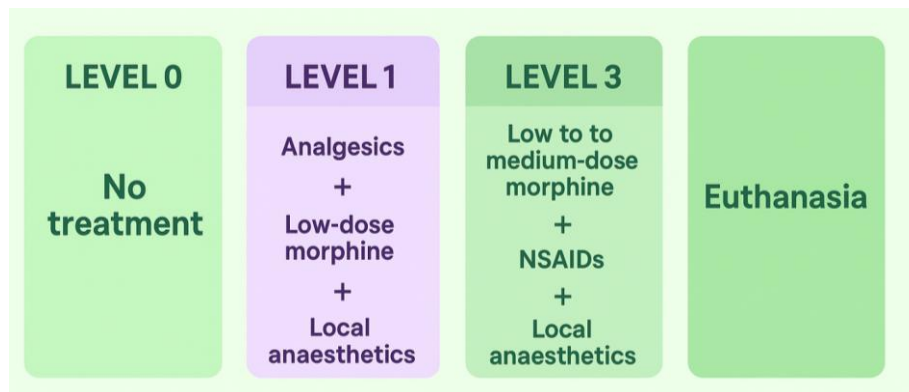
In level 2 stress, representing moderate pain or discomfort, a more comprehensive approach is required. Treatments combine low to medium doses of morphine with NSAIDs to provide systemic analgesia, along with local anesthetics applied directly to the area of manipulation. This

multimodal analgesic strategy addresses both peripheral and central pain pathways, thereby improving the animal's comfort and reducing stress-induced alterations in physiological or behavioral responses.

For level 3 pain, which corresponds to severe or extreme suffering, such as that caused by advanced infectious diseases, cancer, or immunodeficiency, treatment involves medium to high doses of morphine combined with NSAIDs and local anesthetics. These interventions aim to maximize pain relief while maintaining as much physiological stability as possible. In some cases, particularly when pain is intense, progressive, or unresponsive to pharmacological measures, euthanasia becomes the most humane option to prevent prolonged suffering and ensure ethical compliance. Euthanasia should always be conducted using approved methods that are rapid, effective, and minimally stressful for the animal.

The selection and administration of analgesic and anesthetic treatments must consider several factors, including species-specific pharmacokinetics, the nature of the procedure, the expected duration of pain, and the potential interactions with experimental variables. Close monitoring of the animal's behavior, vital signs, and response to therapy is essential to adjust dosages, timing, and administration routes as needed. In addition, all interventions must comply with institutional animal care and use guidelines, national legislation, and international ethical standards, such as the principles of the 3Rs, replacement, reduction, and refinement—which aim to minimize pain and distress while optimizing scientific outcomes.

Overall, a systematic, graded, and preventive approach to pain management not only ensures the welfare of laboratory animals but also enhances the validity and reproducibility of experimental research, creating a framework in which ethical responsibility and scientific integrity are mutually reinforced.



**Figure 10:** Treatments

## CHAPTER 4: RODENTS (RATS, MICE)

### 1. Introduction

Rodents, one of Earth's most diverse and widespread orders of mammals, are fascinating creatures with a significant presence in nature and humans' daily lives. The name "rodent" comes from their defining characteristic: continuously growing incisors that they use to nibble and gnaw on various materials, from plants to objects. This particular adaptation has contributed to the survival and expansion of these animals across a wide range of habitats, from arid deserts to lush forests to crowded urban areas.

#### 1.1 Definition

Rodents are a diverse group of mammals that make up the order Rodentia (Figure 1). They are characterised by a single pair of continuously growing incisors in the upper and lower jaws. Some well-known rodents include mice, rats, squirrels, prairie dogs, porcupines, beavers, guinea pigs, and hamsters. Rabbits, hares, and pikas were once included with rodents but are now considered part of a separate order, Lagomorpha. Nevertheless, Rodentia and Lagomorpha are sister groups that share a common ancestor and form the clade Glires.

Rodents are found on every continent except Antarctica and have adapted to various terrestrial habitats, including anthropogenic ones. Some species are arboreal, fossorial, or semiaquatic. Many rodents have large, well-structured molars with cusps or ridges adapted for grinding food into small pieces. They also have powerful jaw muscles and a mandible pushed forward for gnawing and pulled back during chewing.

Rodents generally have well-developed senses of smell, hearing, and vision. Nocturnal species often have large eyes, and some are sensitive to ultraviolet light. Many rodents have long whiskers, or vibrissae, to scan their surroundings during locomotion. Some rodents have cheek pouches that may be lined with fur.

**Generally speaking, rodents are characterised by:**

**Diversity:** Rodents are members of Rodentia, the most significant order of mammals, comprising over 2,000 species. The most common rodents include mice, rats, hamsters, squirrels, and beavers.

**Adaptability:** Rodents are found worldwide, except for the polar regions. They are incredibly adaptable and can live in various habitats, from deserts to forests to urban areas.

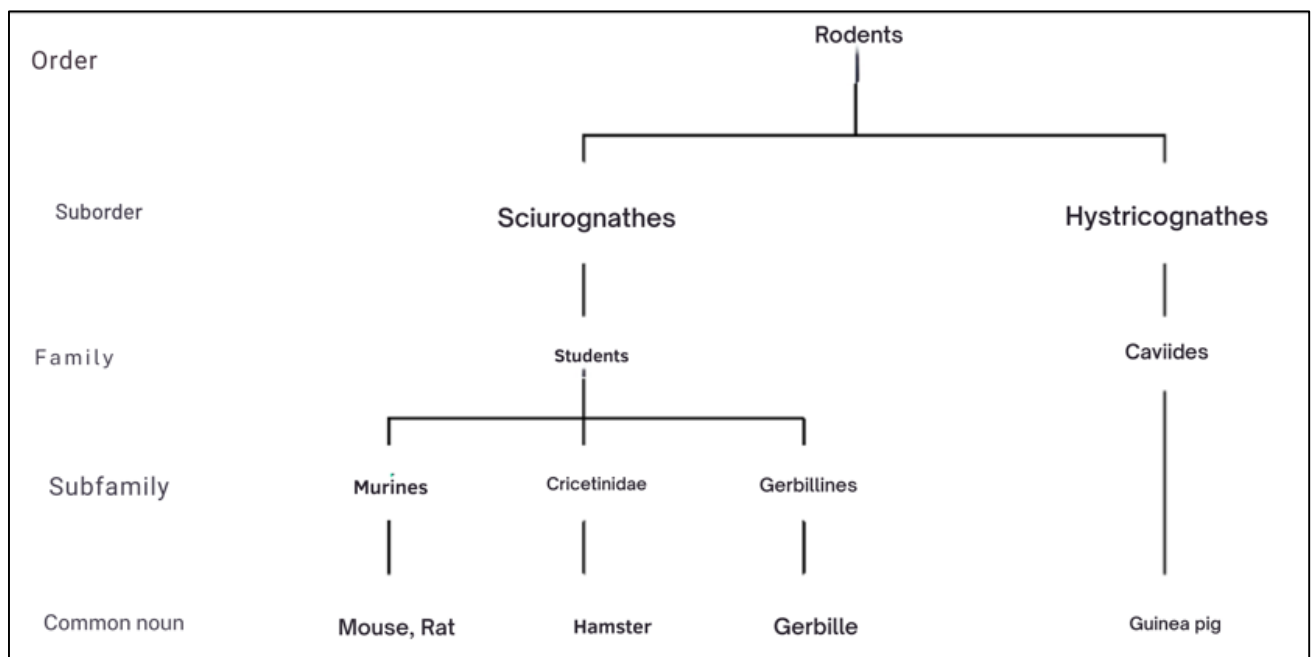
**Diet:** Most rodents are herbivores, primarily on plants, seeds, fruits, and vegetables. However, some are omnivores and also eat insects and other small animals.

**Reproduction :** Rodents generally have a high reproductive capacity. Females can have many litters during their lifetime, with many offspring in each litter. This contributes to the rapid expansion of many rodent species.

**Ecological impact :** it plays an important role in important ecosystems. Rodents are numerous predators. However, rodent species like rats and mice can become pests in urban and agricultural areas.

**Diseases:** Some rodents can transmit diseases to humans, especially when rodent populations proliferate in densely populated environments. Therefore, it is essential to take preventative measures to reduce the risks.

**Pets:** Some rodents, such as hamsters, gerbils, and guinea pigs, are also kept as pets because of their small size and sociable nature.



**Figure 11:** Taxonomy of the order Rodents

## **2. Anatomy**

The anatomy of rodents has distinctive features that distinguish them as an order of mammals. Some of the most common anatomical features are :

**Incisors in continuous growth** the most iconic feature of rodents is their incisors, their front teeth, which grow continuously throughout their lives. These sharp, powerful incisors are adapted for nibbling and gnawing on various materials, including plants, wood, seeds, and objects.

**Specific dentition:** Rodents have distinctive dentition, including incisors. They have 16 teeth, including four incisors (2 on top, two on the bottom), followed by premolars and molars. These teeth are adapted to their diet, which varies depending on the species. For example, herbivores have molars adapted for grinding plants.

**Tail:** Most rodents have an elongated tail, which can vary in length depending on the species. The tail can be bare, covered with hair, or even prehensile in some species.

**Small mammals:** Rodents are generally small, although their size can vary considerably depending on the species. Mice and hamsters can be tiny, while beavers and capybaras can be relatively large.

**Paws** Rodents typically have four legs, each with claws adapted to their lifestyle. These claws can be used for digging, climbing, or grasping objects.

**Hairs:** Most rodents have fur, which can vary in colour, texture, and density depending on the species. Hair can be used for thermal insulation, protection from predators, and communication.

**Eyes and ears:** Rodents generally have small eyes and large ears, although their size can vary depending on the species. Excellent hearing is often essential for detecting predators.

**Specialised stomachs** have specialised stomachs to digest their diet, primarily herbivorous, granivorous, or omnivorous, depending on the species. Some rodents practice caecotrophy, where they consume their faeces to reabsorb nutrients.

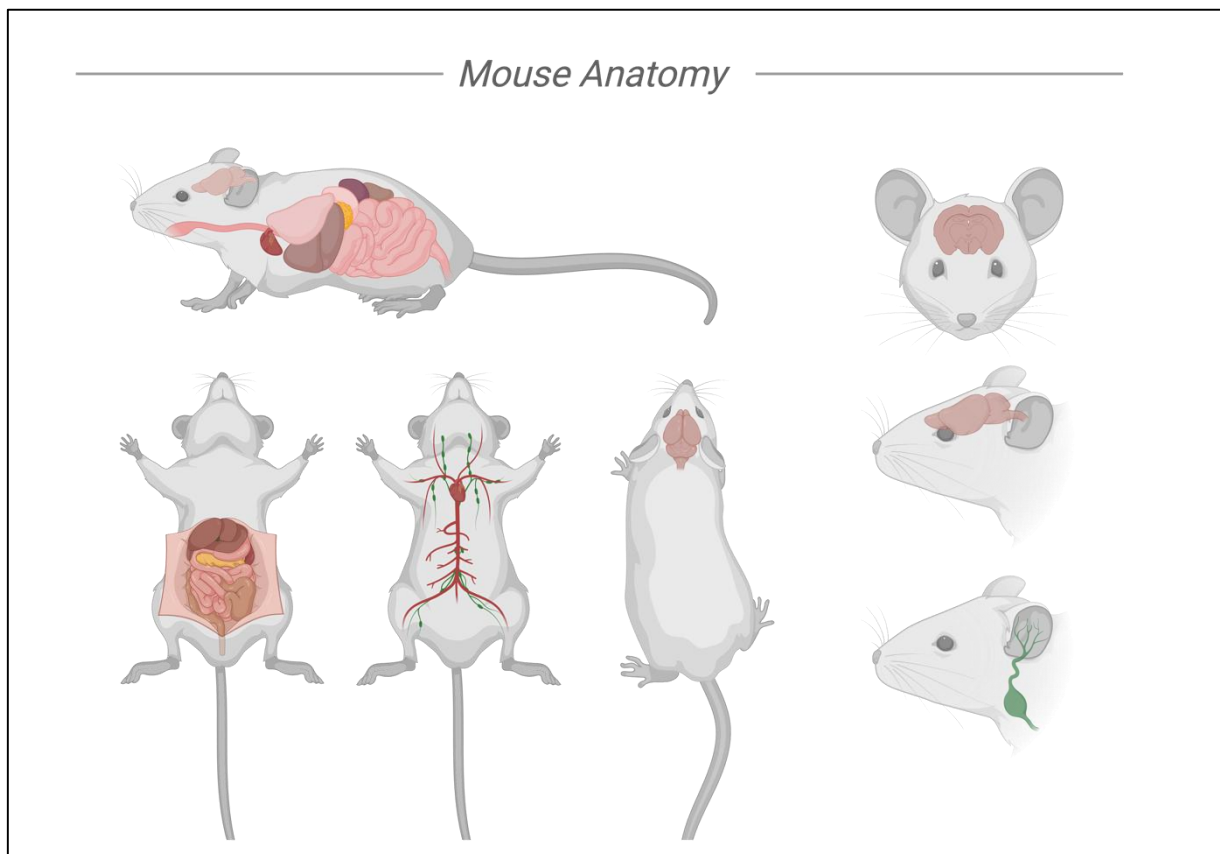
**Reproductive system:** Rodents' gestation period varies from 19 to 35 days, depending on the species. They can have up to 5.4 litters per year, with an average of 5.5 young per litter.

**Nervous system:** The rodent's central nervous system consists of the brain and spinal cord. The brain is divided into several regions, including the cerebrum, cerebellum, and brainstem. The cerebrum is responsible for conscious thought and voluntary actions, while the cerebellum coordinates movement and balance.

**Respiratory system: Rodents' respiratory systems consist** of the lungs, trachea, and bronchi. The lungs are tiny and simple, without lobes or bronchioles. The trachea is short and wide, and the bronchi are few.

**The cardiovascular system:** The cardiovascular system includes the heart, blood vessels, and blood. The heart is small and has four chambers, like those of other blood vessels. It is thin-walled and has a simple structure without valves or capillaries.

These anatomical features are general for rodents, but there is great diversity within this order, with species-specific adaptations to meet their unique dietary needs, habitats, and lifestyles.



**Figure 12:** Rodent anatomy

### 3. The mouse

The mouse "*Mus musculus*" is the most used animal in research, representing approximately 60% of all laboratory animals.

The house mouse or *Mus musculus* is part of:

**The phylum:** vertebrate

**Class:** Mammals

**Order:** Rodents

**Suborder:** Myomorpha

**Family:** Muridae

**Subfamily:** Murinae

The mouse has undergone several genetic manipulations, resulting in several strains that respond to the studies being carried out there.

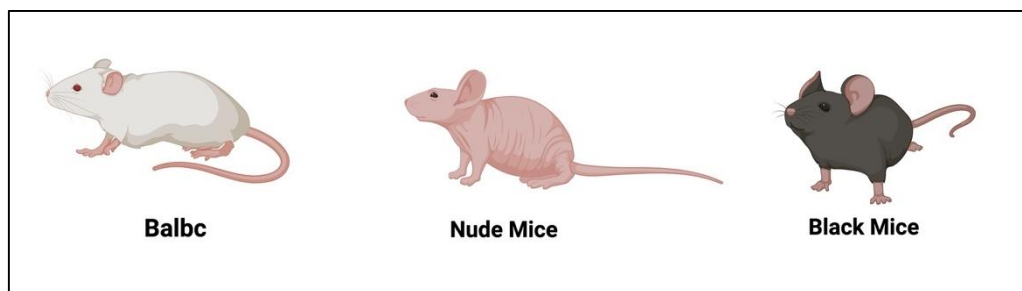
### **3.1 The strains most used in basic and medical-biological research :**

**BALB/c:** This is the albino laboratory mouse, which comes from the domestic mouse by inducing a mutation in the gene that codes for melanin production.

**The Nude Mouse:** This is a hairless "naked" mouse strain characterised by an atrophied thymus or its total absence.

It is most commonly used in cancer research because the absence of T lymphocytes allows the development of pure tumours without contamination by other cells.

C57BL/6 or "Black-6" is the black mouse, a versatile strain used in research on diabetes and obesity. It constitutes the genetic background for the development of transgenic models.



**Figure 13:** The most used mice

### **3.2 Transgenic strains**

Transgenic strains correspond to animals that have received a DNA fragment from another species in their genome or those in which one or more genes have been inactivated.

**The "Doogie" Mouse:** This strain has intelligence that exceeds that of other mice due to the enhanced activity of the brain's N-Methyl-D-Aspartate "NMDA" receptors.

**The oncomouse:** is a mouse that has received a human oncogene, which predisposes it to the development of cancers.

**Knockout mice:** this category includes all mice with one or more inactivated genes.

**Cold-tolerant mice:** These mice are characterised by an exaggerated sensitivity to cold due to the absence of a sodium channel.

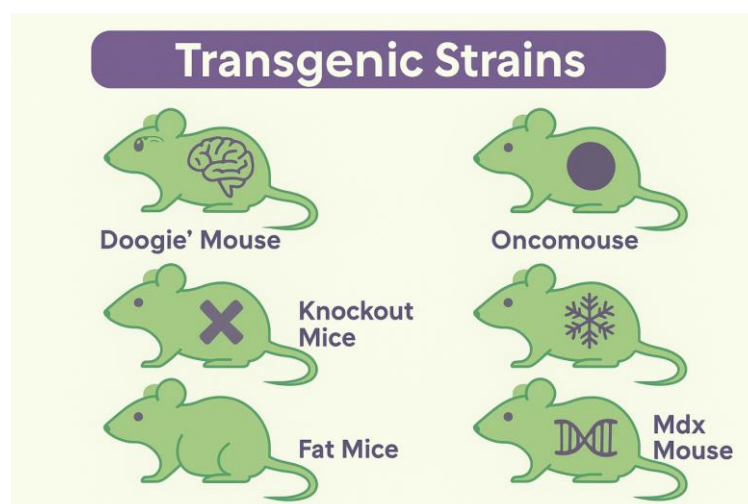
**Fat mice:** are obese mice predisposed to the development of diabetes due to the inactivation of the gene that codes for the enzyme carboxypeptidase E.

**The Mdx mouse:** lacks the gene that codes for dystrophins. It is a model used in the study of Duchenne Muscular Dystrophy.

Using the mouse as a laboratory animal has many advantages: it is small, economical to obtain, house and maintain, and easily manipulated.

The mouse also has the advantage of having a short gestation period and a short lifespan, which makes it possible to carry out tests in a relatively short period that include exposure to a given substance over a significant period of the animal's life (e.g., evaluation of carcinogenic potential and study of tumours).

Adult size is approximately reached at 5 to 6 weeks, but growth is not complete until around 6 months.



**Figure 14:** Transgenic strains

### **3.3 Morphological characteristics**

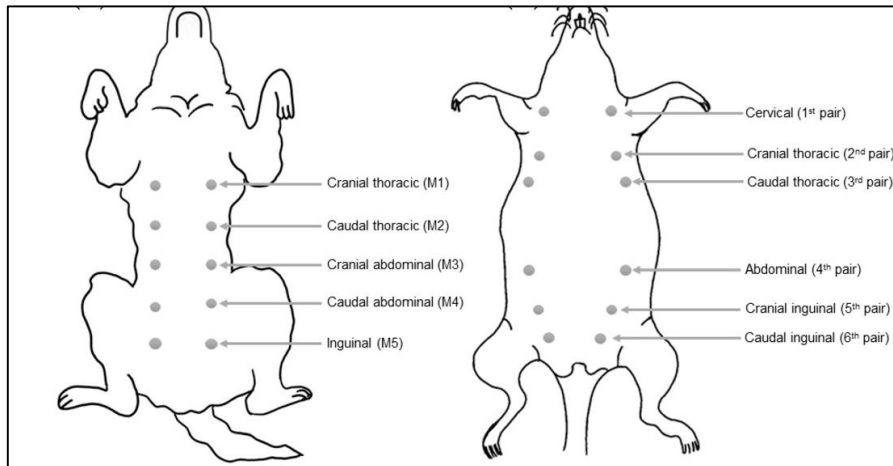
Mice, like rats, exhibit a number of distinctive morphological features that are important for both identification and experimental use. On their forelimbs, mice have four toes, while the hindlimbs bear five toes. The toes are equipped with small, sharp claws that aid in climbing, digging, grooming, and manipulating objects. The limb morphology reflects their burrowing and exploratory behavior, as well as their agility and ability to navigate complex laboratory or natural environments. Both male and female mice possess five pairs of mammary glands, distributed along the ventral surface. These glands are highly developed and extend beyond the typical thoracic and abdominal regions. In some cases, additional or supernumerary teats may be present, which is a naturally occurring variation influenced by genetics or strain-specific characteristics. The mammary tissue in mice is particularly extensive, reaching into the neck region, shoulders, between the thighs, around the genital area, and sometimes along the dorsal line of the back. This extensive distribution is functionally important for lactation, allowing nursing of large litters that may contain six to twelve pups or more depending on the strain.

The skin covering the mammary glands is thin and vascularized, enabling efficient milk production and thermoregulation. During lactation, the mammary glands undergo substantial physiological changes, including proliferation of alveolar tissue, increased vascularization, and expansion of the ductal network, which are critical for supporting the nutritional needs of the offspring. Morphological variations in the mammary glands, such as the presence of extra teats or differences in gland size, are important considerations in breeding programs, developmental studies, and experimental designs that involve reproductive physiology or lactation research.

In addition to the mammary glands and limb morphology, mice exhibit other morphological adaptations relevant for laboratory use. They have a slender body, elongated tail, and delicate skin that allows easy handling but also requires careful attention to prevent injury. The fur coat, which varies in color, density, and length depending on the strain, provides thermal insulation and protects against minor abrasions. Sensory vibrissae on the snout and above the eyes enhance tactile perception, which is essential for navigation, exploration, and social interactions within a cage or experimental environment.

Overall, the morphological characteristics of mice, including limb structure, toe arrangement, and extensive mammary tissue distribution, reflect their reproductive strategy, adaptive behavior, and suitability as model organisms in laboratory research. Knowledge of these traits is essential for

proper animal care, breeding management, and experimental accuracy, particularly in studies related to development, lactation, endocrinology, and toxicology.



**Figure 15:** Topography of the mammary glands in mice

### 3.4 Physiology and reproduction

It varies from strain to strain and depends on nutritional conditions (Table I). Mice use their sense of smell intensively to compensate for their defective vision, particularly in albino strains and create urine-marking patterns in their environment. Their hearing is acute and sensitive to ultrasound.

Because mice have a large body surface area relative to their weight, they are highly susceptible to temperature changes and dehydration. Mice lack sweat glands, and their ability to salivate is limited. They can partially compensate for temperature changes of 20 to 35°C. Mice do not tolerate heat well, and ambient temperatures above 37°C can be fatal.

External factors such as noise, diet, light, and population density are essential in reproduction. Mice reach puberty at 28–49 days and can be bred at 70 days for males and 60–84 days for females.

**Table 1: Mouse physiological data**

General	
Lifetime	1-3 years
Adult weight	
Male	20 – 40g
Female	18 – 35g

Size	12 – 18 cm
Number of chromosomes	40
Food consumption	5 – 8 g/day (15 g/100 g of body weight)
Water consumption	6 – 8 ml/day
Body temperature	37 – 37.2°C
Rectal temperature	38 – 39°C
Heart rate	310 – 840 bpm
Respiratory rate	60 – 220 mpm
Metabolic rate	180 – 550 Kcal/Kg/day
Oxygen consumption	1.69 ml/g/day
Daily production of urine and feces	0.5 – 2 ml of urine 1–1.5 g of feces per day
Reproduction	
Puberty (male and female)	
Gestation	28 – 49 days
Litter (number of pups)	19 – 20 days
Breastfeeding	4-15 litters
Weaning	21 – 23 days 18 - 21 days

#### **4. The rat**

The domestic rat or *Rattus norvegicus*, like the mouse, is part of:

**The phylum:** Vertebrates

**Class:** Mammals

**Order:** Rodents

**Suborder:** Myomorpha

**Family:** Muridae

**Subfamily:** Murinae

The rat is the most widely used species after the mouse. In pharmacotoxicology, the rat has become a species of choice due to its metabolic similarities to humans, its small size, relatively docile nature, short lifespan, and short gestation period.

There are four main varieties of strains: inbred strains (Lewis), non-inbred strains (outbred; Wistar or Sprague-Dawley), first-generation hybrids and mutant strains. More than 1,400 strains and sub-strains are listed and used in biomedical research.

#### **4.1 The most used strains in research:**

*Rattus Norvegicus* is the domestic rat from which all other strains that meet the specific needs of each study were obtained.

**The Wistar Rat:** This is the most widespread strain of laboratory rats, from which other strains have developed. It is used in biological and medical research and is characterised by a broad head, long ears, and a tail shorter than the body.

**The Long-Evans rat:** This strain is a cross between a female Wistar and a wild grey male named after Dr Long Evans, who developed it. It serves as a model for research on obesity and behaviour.

**The BBDP rat (Biobreeding Diabetes Prone):** this strain represents the negative model that develops autoimmunity to type 1 diabetes; it shares many characteristics with humans with type 1 diabetes. Therefore, it is widely used in research conducted on this pathology.

**The Sprague-Dawley rat:** is a strain developed from the Wistar rat. Ideally used in medical research, it is characterised by its calmness and docility.

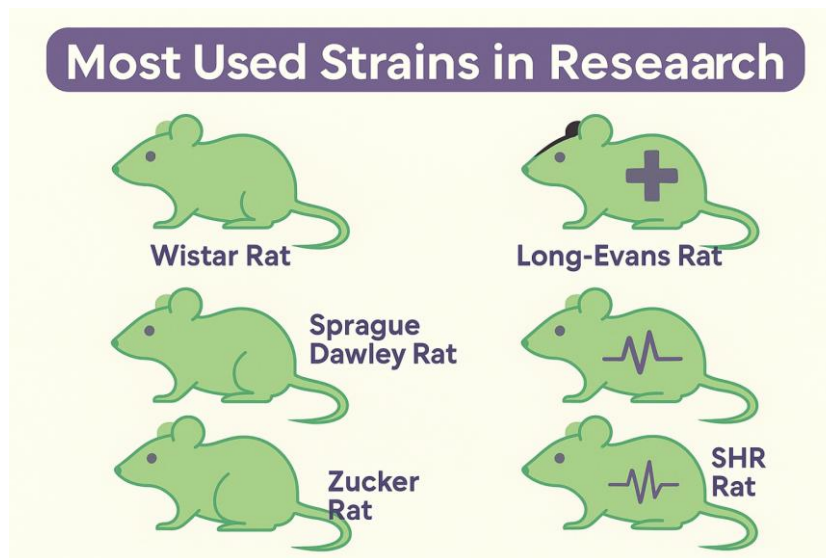
**The Zucker Rat:** is an obese strain with a mutation in the leptin receptor (a hormone that controls satiety). It constitutes the standard model for research on obesity and high blood pressure.

**The Hairless Rat:** This rat is hairless due to a natural mutation not induced by humans. Like the nude mouse, it is characterised by the absence of the thymus, which seriously compromises its immune system.

**The Brattleboro Rat:** This is a rat that presents a natural mutation at the level of the gene that codes for the antidiuretic hormone vasopressin. As a result, the hydromineral balance is disturbed, and the subjects in question are affected by diabetes insipidus, which is characterised by polyuria (abundant urine emission) and polydipsia (excessive thirst).

**The Knockout rat:** This is a strain of rat in which one or more genes have been inactivated, mimicking human diseases. This model is used to study the function of the genes in question and to discover appropriate drugs. The KO rat is used primarily to understand the mechanisms of certain diseases, such as diabetes, Parkinson's, high blood pressure, and Alzheimer's.

**The SHR rat :Spontaneously Hypertensive Inbred Rat:** This strain corresponds to the wild model with natural high blood pressure.



**Figure 16:** The most used strains in research

#### 4.2 Behaviour

The rat is a less gregarious and less photophobic animal than the mouse. It is a nocturnal animal; as soon as the lights are turned off in the pet store, intense activity unfolds in the cages. It gets used to the person handling it and to handling it. The rat feeds during the night phase; the day is devoted to sleep and digestion. The rat has very poor eyesight but a highly developed sense of smell. Males are generally more aggressive than females and can be bitten following fights. However, unlike male mice, male rats do not tend to fight when housed together. Rats also differ from mice in the possibility of housing them individually. However, in the company of their conspecifics, rats are less likely to try to escape and are less stressed.

The rat's intelligence, memory, and rich behavioural repertoire make it a popular subject for studies.

#### 4.3 Physiology and reproduction

Rats are small mammals with unique physiological and reproductive characteristics that are highly relevant in experimental research. One notable feature is their exophthalmic anatomy, meaning their eyes protrude slightly from the orbit. This anatomical trait increases the risk of trauma and ocular drying, particularly during anesthesia or surgical procedures, and necessitates careful protection of the eyes using lubricating ointments or covers to maintain corneal integrity and prevent injury. Despite relatively poor visual acuity in daylight, rats possess enhanced vision in dim light,

especially in pigmented strains, allowing for effective navigation in low-light environments. Their sensory systems are highly developed, with acute hearing and olfactory capabilities. Rats are sensitive to a range of sound frequencies, including ultrasound, which plays a role in communication, environmental perception, and social interactions. The sense of smell is critical for foraging, mate selection, and territory recognition, and olfactory cues are often used in behavioral and neurological experiments.

In terms of reproduction, rats demonstrate early sexual maturity, which is an important consideration for colony management and experimental planning. Puberty is typically reached at 50-60 days of age, with females generally becoming fertile slightly earlier than males. This sexual dimorphism in maturity facilitates controlled breeding programs in laboratory settings. Once sexually mature, rats are capable of reproduction from approximately 65 to 110 days of age, depending on strain and environmental conditions. Female reproductive physiology is characterized by an estrous cycle of approximately 4-5 days, during which hormonal fluctuations regulate ovulation, mating behavior, and reproductive readiness. Estrus can be monitored through vaginal cytology, behavioral cues, and changes in genital morphology.

Male reproductive anatomy includes paired testes located in the scrotum, a penis equipped with a baculum, and accessory glands that contribute to seminal fluid production. Spermatogenesis is continuous once sexual maturity is reached, and males can mate repeatedly over their reproductive lifespan. Female rats possess paired ovaries, oviducts, and a bicornuate uterus, allowing for multiple embryo implantation and high litter sizes. The combination of early sexual maturity, rapid gestation periods (approximately 21-23 days), and large litter sizes makes rats highly suitable for genetic studies, breeding experiments, and developmental biology research.

Physiological characteristics beyond reproduction are also critical. Rats maintain a high metabolic rate, which supports rapid growth and activity levels. Their cardiovascular system features a relatively fast heart rate, while their respiratory system is adapted to high oxygen demands during movement. Thermoregulation is well-developed but requires careful monitoring in laboratory environments to prevent hyperthermia or hypothermia. Nutrition and gastrointestinal function are tightly linked to reproductive success and overall health, as disturbances can lead to reduced fertility or developmental anomalies in offspring.

Overall, the physiology and reproductive biology of rats, including sensory adaptations, exophthalmic eye structure, early puberty, rapid reproductive cycles, and high fecundity, make them invaluable models in biomedical research. Understanding these traits is essential for ethical animal care, experimental design, and interpretation of study results, as physiological and reproductive factors directly influence behavior, response to interventions, and the reliability of experimental outcomes.

**Table 2: Physiological data of the rat**

<b>General</b>	
Average lifespan	2.5 - 3 years
Adult weight	
Male	300 – 900 g
Female	200 – 400 g
Size	12 – 18 cm
Number of chromosomes	42
Food consumption	20 - 40 g/day
Water consumption	10 - 12 ml/100g/day
Body temperature	37.5°C
Rectal temperature	36 – 40°C
Heart rate	250 - 450 bpm
Respiratory rate	70 - 115 mpm
<b>Reproduction</b>	
<b>Puberty</b>	
Male	45 – 46 days
Female	50 days
Gestation	20 - 23 days
Litter (number of pups)	6 - 12 litters
Breastfeeding	22 days
Weaning	21 days

#### **4.4 Pathological dominances**

Pathological dominances, also referred to as pathological dominant genetic traits, are heritable characteristics caused by specific gene mutations that result in observable physiological or morphological abnormalities in rodents. These traits are dominant, meaning that only a single copy of the mutated allele is sufficient to express the pathological condition in an individual. Unlike naturally occurring traits present in all members of a species, pathological dominant traits arise due to spontaneous mutations, selective breeding, or experimental genetic manipulation. They can be transmitted to offspring according to Mendelian inheritance patterns, influencing both experimental outcomes and colony management in laboratory settings.

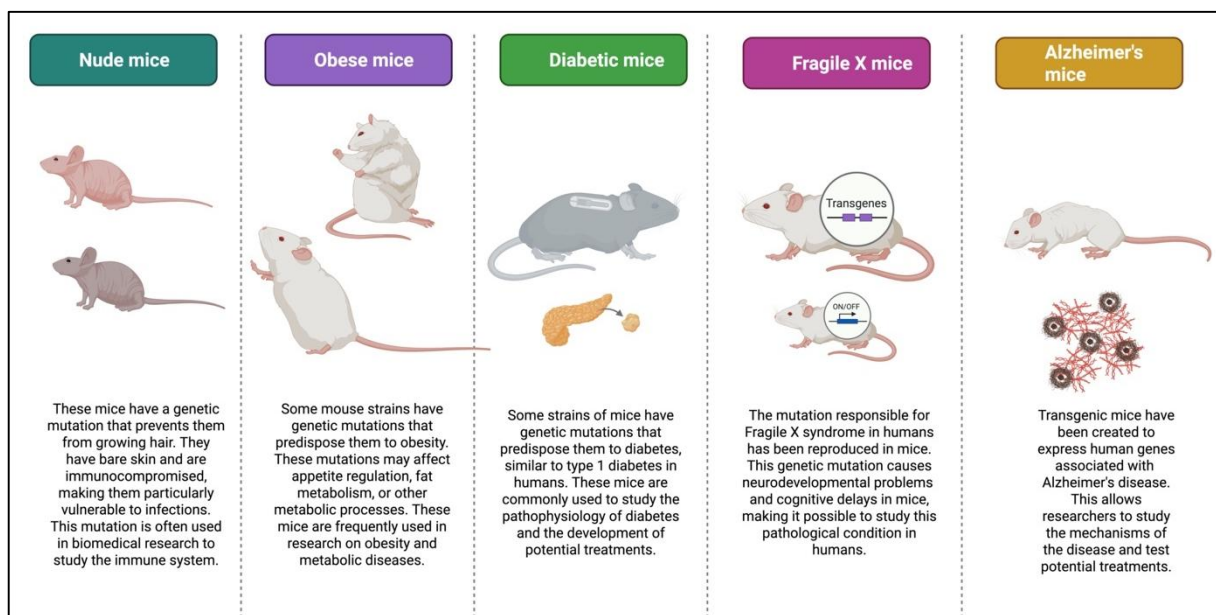
These traits are particularly significant in the context of biomedical research, as they provide models to study genetic diseases, developmental disorders, and pathological mechanisms. For example, certain dominant mutations may lead to neurological dysfunctions, such as motor deficits, seizures, or abnormal behavior, allowing researchers to investigate the molecular and cellular basis of human neurological disorders. Other mutations may affect cardiovascular function, metabolic regulation, or immune responses, serving as preclinical models for heart disease, diabetes, or autoimmune conditions.

Pathological dominances can also impact physical characteristics such as coat color, skeletal morphology, organ size, or sensory function. These visible markers often facilitate the identification and selection of affected individuals for experimental studies. However, they may also pose challenges, including reduced fertility, decreased viability, or increased susceptibility to secondary health issues, which must be carefully monitored to maintain colony health and ensure reliable experimental results.

Examples of pathological dominant traits in rodents include the dilute coat color mutation, which affects pigment distribution, the short-tail phenotype, which alters vertebral development, and certain neurological mutation models, such as those causing ataxia or tremor. Each of these traits provides valuable insight into genetic regulation, tissue development, and disease progression, but their presence requires careful ethical and methodological consideration in research planning.

From an experimental perspective, understanding pathological dominances allows researchers to design controlled studies, predict inheritance patterns, and correlate genotype with phenotype. Maintaining colonies with known dominant traits requires rigorous record-keeping, genetic

screening, and selective breeding strategies to prevent unintended spread or complications. Moreover, these traits emphasize the importance of genetic background in experimental reproducibility, as variations in dominant mutations can influence the manifestation of disease phenotypes or response to therapeutic interventions.



**Figure 17: Genetically Modified Mouse Models for Biomedical Research**

It is important to note that this pathological dominance in rodents is generally the result of intentional genetic manipulation in the laboratory to study specific human diseases. These animal models are used in biomedical research to understand diseases better, develop potential treatments and test new therapeutic approaches. The rodents in these studies are subject to strict ethical regulations to ensure their welfare.

## **CHAPTER 5: THE LAGOMORPHS**

### **1. Introduction**

Lagomorphs are an order of mammals characterised by their herbivorous diet and unique physical makeup. Comprised of two prominent families, Leporidae (such as rabbits and hares) and Ochotonidae (such as pikas), lagomorphs are often associated with a variety of habitats, ranging from mountainous environments to grasslands to arctic regions.

Their name, "lagomorpha," derives from the ancient Greek word for "hare-like." These animals are distinguished by their large, prominent incisors, essential for their diet and primarily based on plants. Their ability to digest plant fibre is remarkable, and their specialised digestive system allows them to extract nutrients from even the most difficult-to-digest plants.

Lagomorphs occupy diverse ecological niches and play a crucial role in many ecosystems as herbivores. Their reproduction is also interesting, characterised by rapid reproduction and considerable proliferation.

Despite their apparent cuteness and popularity as pets, lagomorphs play an essential biological role in many ecosystems, and the study of their behaviour, physiology, and ecology continues to attract the attention of researchers.

### **2. Lagomorph in animal experimentation**

Lagomorphs, including rabbits and hares, are commonly employed in animal experimentation due to their anatomical, physiological, and reproductive characteristics, which make them suitable for a wide range of scientific studies. They serve as valuable models for investigating disease mechanisms, pharmacological interventions, toxicological responses, and the safety of chemicals and cosmetic products. Their relatively large size compared to rodents facilitates certain procedures, such as repeated blood sampling, surgical interventions, and administration of substances, while their physiological systems exhibit responses that are often relevant to human biology.

In biomedical research, rabbits are frequently used for immunology studies, including antibody production, vaccine development, and autoimmune disease research. Their reproductive characteristics, such as induced ovulation and high fertility, make them suitable for studies involving

reproductive biology and developmental processes. Additionally, their gastrointestinal physiology, which includes cecotrophy and a sensitive digestive system, provides a model for studies of nutrition, metabolism, and gastrointestinal disorders. Hares are less commonly used but may serve as models in ecological or comparative physiology studies.

The use of lagomorphs in experimentation requires specialized training for personnel involved in the design and execution of experiments. Such training emphasizes ethical considerations, regulatory compliance, and technical expertise to ensure that procedures are carried out responsibly and humanely. Technicians and researchers are trained to minimize animal distress, perform procedures efficiently, and monitor animals for welfare throughout the study.

Ethical reflection is central to animal experimentation. Researchers are required to adhere to international guidelines and local regulations, ensuring that the use of animals is justified by the scientific value of the research. The ethical framework also involves a critical evaluation of experimental design, questioning whether the objectives can be achieved through alternative models, such as in vitro systems or computer simulations.

The 3R principle—replacement, reduction, and refinement—guides all aspects of animal research. Replacement encourages the use of non-animal methods wherever possible, reduction aims to use the minimum number of animals necessary to achieve statistically valid results, and refinement focuses on modifying procedures to minimize pain, stress, and suffering. Applying these principles in studies involving lagomorphs ensures that experiments are scientifically rigorous while maintaining high standards of animal welfare.

### **3. General anatomy of lagomorphs**

Lagomorphs like rabbits and hares are running animals with specific anatomical characteristics. The essential anatomical aspects of lagomorphs:

Lagomorphs, including rabbits and hares, are small to medium-sized herbivorous mammals with anatomical features that reflect their evolutionary adaptation to a **cursorial and herbivorous lifestyle**. These animals are highly specialized for rapid locomotion, predator avoidance, and efficient nutrient extraction from a plant-based diet. Their anatomy is therefore characterized by a combination of structural, sensory, and physiological adaptations that work together to support survival in diverse environments.

The ears of lagomorphs are extremely large relative to the size of their heads, often equaling or exceeding head length. These ears are highly vascularized and contain numerous muscles that allow precise movement, rotation, and orientation towards sound sources. The large surface area of the ears is not only critical for detecting predators at a distance but also plays a significant role in thermoregulation, as blood flow through the ear surfaces can dissipate excess heat. In addition, the ears facilitate communication, with subtle changes in position conveying signals of alertness, fear, or submission to conspecifics. The combination of auditory acuity and mobility ensures that lagomorphs maintain high situational awareness, which is essential given their vulnerability as prey animals.

The limbs of lagomorphs are specialized for leaping and rapid sprinting. The hind legs are long, muscular, and powerful, with elongated metatarsals and a flexible ankle joint that maximizes stride length and propulsion. This structural arrangement allows for sudden bursts of speed and high jumps, which are critical for escaping predators in open habitats. The forelimbs are shorter but strong and agile, serving primarily in stabilization, grooming, and manipulating food items. The paw pads are thick and cushioned, protecting against repeated impact during rapid movement, while sharp claws provide traction on various substrates. This limb morphology, combined with a lightweight skeletal structure, optimizes both speed and endurance during locomotion.

The digestive system of lagomorphs is highly adapted to a herbivorous diet that is rich in fiber but low in easily digestible nutrients. The gastrointestinal tract contains a large cecum that houses a dense microbial population capable of fermenting cellulose into volatile fatty acids, providing essential energy sources. Lagomorphs also exhibit a unique behavior known as caecotrophy, in which they produce and re-ingest soft fecal pellets called cecotropes. This process enables the absorption of nutrients, including B vitamins and microbial proteins, that were not fully extracted during the first passage through the digestive tract. The stomach and intestines are elongated to increase transit time and maximize nutrient extraction, and the colon is specialized to separate indigestible fiber from nutrient-rich material destined for caecotrophy. This complex digestive adaptation makes lagomorphs particularly sensitive to dietary changes, stress, and gastrointestinal disorders such as stasis or dysbiosis.

The respiratory system of lagomorphs is similar to that of other small mammals but is supported by a large and flexible abdominal cavity. The diaphragm separates the thoracic and abdominal cavities and plays a critical role in facilitating both inspiration and expiration. This arrangement

allows for efficient ventilation of the lungs during periods of high metabolic demand, such as rapid running. The ribcage is relatively rigid, providing structural protection to the thoracic organs while the abdominal muscles assist in respiration, particularly during physical exertion. The nasal passages are long and well-vascularized, allowing for humidification and warming of inhaled air, which is important for maintaining respiratory efficiency and overall health.

The circulatory system is designed to support the high metabolic rate of lagomorphs. The heart is small relative to body size but exhibits a high resting rate, typically between 180 and 250 beats per minute. This rapid heart rate facilitates efficient oxygen delivery to muscles during periods of activity. Arterial and venous networks are highly developed, ensuring rapid circulation throughout the body. Additionally, the lymphatic system plays a role in immune surveillance and fluid balance, which is particularly important given the exposure of lagomorphs to pathogens in their environment and diet.

The reproductive system of male lagomorphs consists of two hairless, retractable testicles located in a scrotum, which allows for regulation of testicular temperature, optimizing spermatogenesis. The penis is equipped with a baculum, which provides structural support during copulation. Female lagomorphs have a vulva located just above the anus, two ovaries, and two oviducts that terminate in a bifid uterus. This unique uterine structure enables the female to carry multiple embryos simultaneously, reflecting the species' high reproductive potential and the evolutionary pressure for rapid population growth. Ovarian activity is influenced by environmental cues and social interactions, with induced ovulation triggered by copulatory stimuli rather than occurring spontaneously. These reproductive adaptations make lagomorphs valuable models for studies in reproductive physiology, endocrinology, and developmental biology.

The dentition of lagomorphs is highly specialized for herbivory. They possess continuously growing incisors that are well-suited for cutting through tough plant material, as well as premolars and molars designed for grinding fibrous foods. The absence of prominent canines allows for efficient processing of plant matter, and the dental formula reflects the need for precise occlusion and wear to prevent overgrowth. Dental health is critical, as malocclusion can lead to severe feeding difficulties, weight loss, and secondary health issues. The jaw musculature is well-developed, supporting lateral and vertical movements necessary for mastication.

The muzzle and nose are highly developed sensory structures. The elongated snout enhances the sense of smell and provides dexterity for manipulating food items and exploring the environment.

Vibrissae around the snout detect tactile stimuli, which assists in navigation and foraging, particularly in low-light conditions. The lips are mobile and sensitive, facilitating selective feeding and precise handling of plant materials.

Lagomorphs exhibit remarkable visual adaptations. They are primarily crepuscular, active during dawn and dusk, and possess large eyes positioned laterally, providing an almost panoramic field of vision of approximately 190 degrees per eye. This wide visual field is crucial for predator detection and spatial orientation in open habitats. The retina contains a high density of rod cells, enhancing low-light vision, while color vision, although limited compared to primates, allows discrimination of contrasts in the environment. Combined with acute auditory and olfactory senses, these adaptations ensure that lagomorphs maintain high environmental awareness and responsiveness to potential threats.

#### **4. The rabbit**

The laboratory rabbit descends from the so-called “European” rabbit, *Oryctolagus cuniculus*; it is part of the Leporidae family.

The rabbit is a shy animal that likes peace. It is always worried.

It reproduces quickly; this high productivity is linked to permanent ovulation induced by short matings, gestation and lactation.

##### **4.1. Behaviour**

The rabbit is a fearful animal. It can sometimes be nervous in its cage and taps the ground with its paw. It can bite and scratch, and its hind legs are well-relaxed. During mating, the female must visit the male in his cage. Newborns are blind and hairless and open their eyes after 7-10 days. They leave the weaned cave around 18 days and leave the nest at 23-25 days.

##### **4.2 Physiology**

The rabbit exhibits two particularly notable physiological characteristics that are crucial for understanding its biology and susceptibility to certain pathologies. The first is its reproductive physiology, specifically induced ovulation. Unlike spontaneous ovulators, female rabbits (does) release eggs only in response to specific stimuli, such as copulatory behavior or mechanical stimulation during mating. This feature has significant implications for reproductive studies, breeding man-

agement, and experimental design, as it influences fertility timing, hormonal cycles, and reproductive success. Understanding this characteristic is essential when using rabbits as models in reproductive biology, endocrinology, or pharmacological research.

The second distinctive feature is the rabbit's feeding behavior, which is dominated by caecotrophy. Rabbits produce two types of fecal pellets: hard feces, which are excreted, and soft cecotropes, which are re-ingested directly from the anus. This behavior allows rabbits to recycle nutrients, particularly B vitamins and microbial proteins, and maintain optimal digestive efficiency. Caecotrophy makes the rabbit's gastrointestinal system highly specialized and particularly sensitive to digestive pathologies, such as dysbiosis, gastrointestinal stasis, and enteritis. Any disturbance in diet, microbiota composition, or stress levels can rapidly lead to serious health complications, highlighting the importance of careful dietary management and monitoring in both husbandry and experimental contexts.

Together, these physiological traits, the reproductive mechanism of induced ovulation and the unique digestive strategy of caecotrophy, define the rabbit's biological characteristics, experimental considerations, and susceptibility to disease, making it a valuable yet sensitive animal model for research in reproduction, nutrition, metabolism, and gastrointestinal health.

**Table 3: Physiological data of the rabbit**

<i>General</i>	
<i>Longevity</i>	<i>5 - 13 years old</i>
<i>Adult weight</i>	
<i>Male</i>	<i>4 - 5 kg</i>
<i>Female</i>	<i>4 - 6 kg</i>
<i>Size</i>	<i>35 - 45 cm</i>
<i>Number of chromosomes</i>	<i>44</i>
<i>Food consumption</i>	<i>50 g/kg/day</i>
<i>Water consumption</i>	<i>50 ml/kg/day</i>
<i>Body temperature</i>	<i>39.6 – 40.1°C</i>
<i>Rectal temperature</i>	<i>38.5 – 40°C</i>
<i>Basal metabolism</i>	<i>100 cal/kg/day</i>
<i>Gastrointestinal transit time</i>	<i>4 – 5 hours</i>

<i>Heart rate</i>	<i>130 - 325 bpm (beats per minute)</i>
<i>Respiratory rate</i>	<i>30 – 60</i>
<i>Blood volume</i>	<i>55 – 70 ml/Kg</i>
<i>Reproduction</i>	
<i>Puberty</i>	
<i>Male</i>	<i>6 – 10 months</i>
<i>Female</i>	<i>6 – 9 months</i>
<i>Gestation</i>	<i>29 - 35 days</i>
<i>Number of litters (number of pups)</i>	<i>5 to several litters per year</i>
<i>Weaning</i>	<i>4 – 6 weeks</i>

### **4.3 Areas of use**

The areas of use of rabbits are:

**Skin irritation test:** Rabbits assess skin irritation after applying certain products, such as cosmetics.

**Draize test:** This test assesses the skin corrosion caused by chemicals. Rabbits are restrained to prevent them from looking at the substances or applying them to their shaved skin.

**Blood samples:** Rabbits sometimes extract blood for pharmacology and toxicology studies.

Rabbits are used in immunotechnology for various applications, including producing polyclonal antibodies and immunotherapy research.

**Production of polyclonal antibodies:** Rabbits are used to produce polyclonal antibodies, molecules capable of recognising multiple epitopes of an antigen. The amount of immunogen needed to complete an immunisation protocol is 800-1000 µg per rabbit. These polyclonal antibodies can be used for various applications, such as Western blotting, ELISA, immunostaining, and immunoprecipitation assays.

**Improving vaccination uptake:** Rabbits are used to optimise the immune status of vaccines, helping to improve vaccine uptake and boost the animal's overall immunity.

**Evaluation of the effectiveness of immunotherapies:** Rabbits can be used to test the efficacy of new immunotherapies by assessing the activation of CD4 T lymphocytes after treatment with specific molecules, such as cGAMP (a molecule called "2'3'-cyclic GMP-AMP", which is an intracellular second messenger produced in response to cytosolic double-stranded DNA).

## CHAPTER 6: OTHER ANIMAL MODELS USED IN SCIENTIFIC RESEARCH

Animal models other than rodents and lagomorphs are crucial in scientific research, providing diverse physiological and genetic information. Various species, including mammals, birds, reptiles, amphibians, and invertebrates, are used to study diseases, drug efficacy, and toxicology. This diversity allows for a better understanding of biological processes and the development of treatments.

### 1. Mammalian models

**Dogs and pigs:** Canine organoids study cancers and inflammatory responses, while porcine models are used for viral research, such as H1N1 (Zdyrski et al., 2024).

**Primates:** Non-human primates play a critical role in understanding complex diseases and developing vaccines, particularly in pandemic-related studies (Domínguez-Oliva et al., 2023).

Non-mammalian models

**Zebrafish:** This aquatic model is increasingly used in genetic and developmental studies due to its transparent embryos and rapid development (Domínguez-Oliva et al., 2023).

**Invertebrates:** Species such as *Drosophila* (fruit flies) and *Caenorhabditis elegans* (nematodes) are essential for genetic research and understanding fundamental biological processes (“Animals: Pioneers or Victims of Experimental Biology?”, 2022).

Although these alternative models provide valuable insights, translating the findings into human applications remains challenging due to species-specific physiological and genetic differences (“Animals: Pioneers or Victims of Experimental Biology?” 2022).

Besides rodents and lagomorphs, several other animal models are widely used in scientific research. Primate models, such as rhesus macaques and chimpanzees, are beneficial for studying complex behaviours and cognitive functions due to their genetic similarity to humans, making them ideal for human disease research and treatment. Canine models, including various dog breeds, are used to study diseases such as cancer and cardiovascular disease, taking advantage of their similar pathological profiles to humans. Feline models, such as domestic cats, are also used to study diseases such as HIV and cancer, offering advantages in size and maintenance. In addition,

porcine models are used for research on cardiovascular disease and diabetes. In contrast, sheep models contribute to respiratory and neurological disorders studies, benefiting from human physiological similarities. These various models improve our understanding of human health and disease.

## **2. Fish Models**

Fish models, such as zebrafish and medaka, are used in scientific research to study human diseases such as cancer, cardiovascular disease, and neurological disorders. Their small size, rapid reproduction, and transparent embryos make them an attractive model for understanding human health and disease.

The use of fish in scientific research is increasing worldwide, both due to the rapid expansion of the fish farming industry and growing awareness of issues regarding the human use of mammalian models in basic research and chemical testing (Schaeck et al., 2013).

## **3. Avian Models**

Avian models, such as chickens and turkeys, are used in scientific research to study human diseases such as cancer, developmental biology, and immunology. Their unique characteristics, such as egg-laying, make them an attractive model for understanding human physiology and disease.

Furthermore, avian species can be used to study the aetiology of human ovarian cancer and other human diseases, such as disorders related to abnormal lipid metabolism and unique mechanisms of cholesterol biosynthesis and transport (Song & Han, 2011).

## **4. Goat Models**

Goat models are used in scientific research to study human diseases such as tuberculosis, brucellosis, and other infectious diseases. Their human-like size and physiology make them an attractive model for understanding human health and disease.

## **5. Sheep Models**

Sheep models are used in scientific research to study human diseases such as respiratory diseases, cardiovascular diseases, and neurological disorders. Their similar size and physiology to humans make them an attractive model for understanding human health and disease.

## **6. Pig Models**

Pig models are used in scientific research to study human diseases such as cardiovascular disease, diabetes, and cancer. Their similar size and physiology to humans make them an attractive model for understanding human health and disease.

## **7. Feline Models**

Feline models, such as domestic cats, are used in scientific research to study human diseases such as HIV, cancer, and neurological disorders. Their small size and relatively low maintenance requirements make them attractive models for understanding human physiology and disease.

## **8. Canine Models**

Canine models are used in scientific research to study human diseases such as cancer, cardiovascular disease, and neurological disorders. Their genetic similarity to humans and similar pathological profiles make them an attractive model for understanding human health and disease.

Canine models are particularly useful because they resemble advanced castration-resistant human diseases with osteoblastic bone metastases. Animal models of prostate cancer are essential for understanding its pathogenesis and developing treatments (Simmons et al., 2014).

There are several hundred isolated dog populations, and each has significantly reduced genetic variation compared to humans, simplifying disease mapping and pharmacogenomics (Rowell et al., 2011).

## **9. Primate Models**

Primate models, such as rhesus macaques and chimpanzees, are used in scientific research to study human diseases and develop new treatments. They are handy for studying complex behaviours and cognitive functions. Their genetic similarity to humans makes them an interesting model for understanding human physiology and disease.

Non-human primates (NHPs) represent clinically relevant animal models used in studies of the aetiology and treatment of human diseases. These models are appropriate for research interests as diverse as the aetiology and treatment of infertility, the development of contraceptives or the evaluation of cell or tissue-based disease therapies using phenotypes derived from embryonic stem cells (Wolf et al., 2008).

Non-human primates are not only closely related to humans in terms of taxonomic status but also possess a developed, sophisticated prefrontal cortex (PFC) and exhibit addiction patterns similar to those of humans (Wang, 2014 For decades, n.

Nonhuman primates have been used as animal models to study cognitive changes associated with ageing and age-related diseases. Using nonhuman primates for ageing studies has many advantages, including examining nonspatial visual cognitive processes and using operational behavioural tasks similar to those used in humans (MI & Gp, 2004).

## **10. Rabbit Models**

Rabbit models are used in scientific research to study human diseases such as cardiovascular disease, cancer, and neurological disorders. Their similar size and physiology to humans make them an attractive model for understanding human health and disease.

Various animal models have been used to study cardiovascular diseases, contributing to our understanding of their pathophysiology and treatment. This review summarizes the transgenic rabbit models developed to study cardiovascular diseases (Peng, 2012).

In addition, rabbits help study orthopaedic surgery, cardiovascular surgery, and neoplastic diseases. Rabbit models have contributed to mechanistic studies of human diseases and the development of therapeutic compounds, devices, or techniques (Shiomi, 2009).

These findings may interest researchers in related fields and contribute to strengthening translational research on cardiovascular diseases. Efforts have been made through a doctoral research

program to exploit the advantages of using rabbits for cardiac imaging research and overcome technical barriers (Feng et al., 2014).

### **11. Donkey Models**

Donkey models are used in scientific research to study human diseases such as cancer, cardiovascular disease, and neurological disorders. Their human-like size and physiology make them an attractive model for understanding human health and disease.

### **12. Horse Models**

Horse models are used in scientific research to study human diseases such as osteoarthritis, laminitis, and other musculoskeletal disorders. Their similar size and physiology to humans make them an attractive model for understanding human health and disease.

### **13. Cattle Models**

Bovine models are used in scientific research to study human diseases such as bovine spongiform encephalopathy (BSE) and other infectious diseases. Their similar size and physiology to humans make them an attractive model for understanding human health and disease.

### **14. Xenopus Models**

Xenopus models, such as the African clawed frog, are used in scientific research to study human diseases such as cancer, developmental biology, and immunology. Their unique characteristics, such as their aquatic environment, make them an attractive model for understanding human physiology and disease.

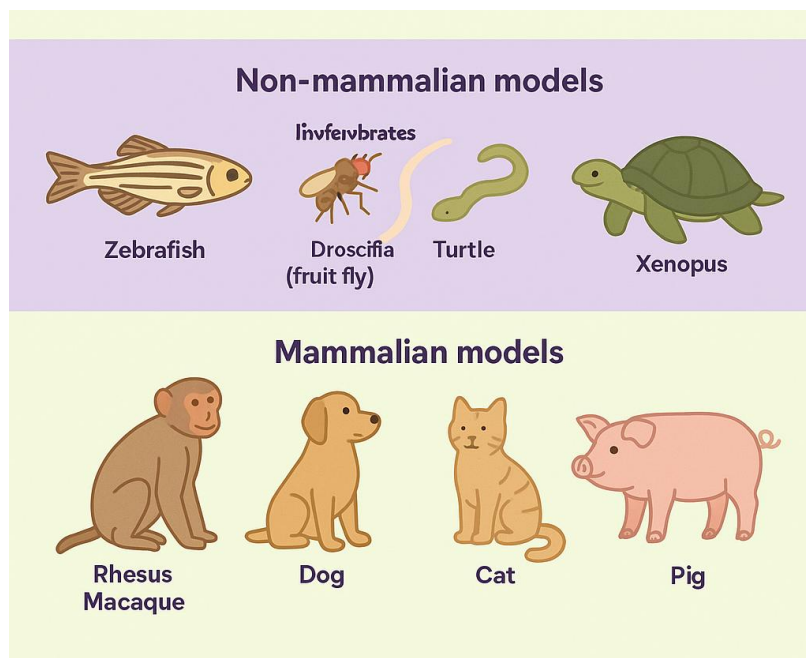
### **15. Invertebrate Models**

Invertebrate models, such as fruit flies, nematodes, and honeybees, are used in scientific research to study human diseases such as cancer, neurological disorders, and developmental biology. Their

simple nervous systems and rapid reproduction make them attractive models for understanding human physiology and disease.

## 16. Turtle Models

Turtle models are used in scientific research to study human diseases such as cancer, developmental biology, and immunology. Their unique characteristics, such as their slow growth rate, make them an attractive model for understanding human physiology and disease.



**Figure 18:** Other animal models used in scientific research

## CHAPTER 7 : EXPERIMENTAL TECHNIQUES

### 1. Introduction

Animal experimentation is a common practice in scientific research. It aims to acquire fundamental knowledge about biological processes and diseases and develop new medical treatments. Experimental techniques are varied and apply a rigorous and ethical approach to ensure the welfare of the animals used and the validity and relevance of the results obtained.

Animal experiments can cover many fields, such as medicine, biology, pharmacology, and toxicology. Animal models, such as rodents, fish, primates, and other species, are selected based on the study's specific objectives. Experimental techniques can be non-invasive, such as behavioural observation, or invasive, involving surgical procedures, tissue sampling, or genetic manipulation. Experimental planning is crucial for designing robust and ethically justifiable studies. Researchers must adhere to strict animal ethics standards, ensuring that experimental protocols minimise animal suffering and use the minimum number of animals necessary to obtain meaningful results.

### 2. Control of biological parameters and validation of experiments

Animal experiments require controlling biological parameters and validating experiments to ensure reliable and ethical results. **The key aspects include:**

#### 2.1 Biological Parameters

##### 1. Standardisation of procedures

Establish standardised experimental protocols to minimise variation (e.g. environmental conditions, time of day, diet).

##### 2. Selection and characterisation of animal models

Choose models with well-understood genetic, physiological, and behavioural characteristics, ensuring relevance to the studied condition.

##### 3. Physiology

Understand the animals' physiology to assess health and responsiveness to treatments or experimental conditions.

4. Anatomy and biology  
Consider specific anatomical and biological traits, and factor in ethology (behavioural responses to stimuli).
5. Health  
Monitor general health, nutrition, and maintenance to avoid unintended result variability.
6. Genetic  
Account for genetic variants which may affect responses to treatments or experimental conditions.
7. Environment  
Control environmental variables (temperature, humidity, light, safety) to reduce bias and variability.
8. Control of experimental variables  
Identify and manage all variables influencing the experiment (e.g. age, sex, diet, housing).
9. Measures of interindividual variability  
Acknowledge natural variability among individuals; determine adequate sample sizes to ensure statistical significance.
10. Validation of evaluation methods  
Ensure methods measuring biological parameters are validated (reproducibility, equipment calibration, use of reference methods).
11. Ethical control  
Submit experiments for ethical review; committees evaluate justification, animal welfare, and responsible resource use.
12. Appropriate statistical analysis  
Select and apply suitable statistical tests to reliably interpret the data.
13. Transparency and publication of results  
Publish complete methods, results, and analyses; include randomisation and allocation concealment practices.
14. Use of witnesses and control groups  
Employ appropriate controls to enable valid comparisons with experimental groups.
15. Replication of studies  
Allow independent reproduction of studies to confirm reliability.

## **2.2 Experimental Validation**

1. Internal validity  
Ensure accuracy in measuring what is intended; minimise biases and systematic errors.
2. Reproducibility  
Design experiments to be reproducible by others using the same methods.
3. Control of confounding variables  
Identify and manage all variables that could distort outcomes.
4. Measure of variability  
Address measurement variability (instrument reliability, inter-individual differences, environmental effects).
5. Ethical control (again)  
Adhere to moral principles and ethical guidelines, respecting animal welfare and regulatory standards.
6. Validation of measuring instruments  
Demonstrate that instruments measure what they are intended to.
7. Representative sampling  
Use representative samples to avoid biases; choose sampling strategies carefully.
8. Appropriate statistical analysis (repeated)  
Use statistical tests aligned with the study design and data characteristics.
9. Predefined success criteria  
Set criteria for success before starting the experiment to avoid result-based bias.
10. Transparency and documentation  
Keep detailed records of every experiment stage to enable verification and reproducibility.

## **3. Techniques and methodology to follow during the different phases of animal experimentation**

Animal experimentation involves several phases, from study design to analysis of results. Each phase requires careful planning, precise execution, and ongoing ethical review. The key steps in the techniques and methodology to follow during the different stages of animal experimentation are:

### **3.1 Planning Phase**

**Definition of objectives:** Clarify the experiment's objectives to determine the type of experiment, the animal models needed, and the parameters to be measured.

**Experimental design:** Develop a detailed experimental protocol, including experimental and control groups, measurements to be taken, and variables to be controlled.

**Ethical considerations:** Evaluate the experiment's ethics, including the justified use of animals, respect for animal welfare, and approval by ethics committees.

### **3.2 Execution Phase**

**Animal handling:** Carefully handling animals, minimising stress and pain. Anesthesia, restraint, and handling techniques must be adapted to reduce suffering.

**Administration of treatments:** This step involves administering treatments, such as drugs, chemicals, or surgical procedures, according to the experimental protocol.

**Variable control:** Maintain constant conditions to control external variables and minimise experimental bias.

**Data collection:** Follow established protocols to collect data systematically and accurately. Carefully document all observations.

**Animal welfare monitoring:** Continuously monitor animal welfare, assessing their physical and behavioural health.

### **3.3 Analysis Phase**

**Data analysis:** Apply appropriate statistical methods to analyse the collected data, validly comparing experimental and control groups.

**Interpretation of results:** Interpret the results with caution, considering the internal and external validity of the experiment.

### **3.4 Reporting Phase**

**Report writing:** Write a detailed report describing the methods, experiment results and conclusions of the

**ing and sharing:** Submit the report for peer review and share the results transparently, thereby contributing to the advancement of scientific knowledge.

Considerations throughout the process:

**Continuous assessment:** Regularly re-evaluate protocols to improve methods and minimise animal suffering.

**Training and skills:** Ensure adequate training for those involved in animal experimentation to ensure appropriate and ethical practices.

**Compliance with regulations:** Strictly follow local and international laws regarding the use of animals in research.

#### **4. Techniques used for the administration of substances**

There are several techniques for administering substances to laboratory animals. The most common techniques are:

**Oral administration:** Substances can be administered orally by mixing the substance with food, water, or treats or using a gastric or nasogastric tube to administer the substance directly into the stomach.

**Administration by injection:** Substances may be administered by injection, including intravenous, intramuscular, subcutaneous, or intraperitoneal injection.

**Administration by inhalation:** Substances can be administered using an inhalation chamber or mask.

**Topical administration:** Substances can be applied directly to the skin or mucous membranes using creams, gels or solutions.

**Rectal administration:** Substances can be administered rectally using suppositories or enemas.

**Table 4: Summary of Substance Administration Techniques**

<b>TECHNIQUE</b>	<b>METHOD OF ADMINISTRATION</b>	<b>NOTES/Common Application</b>
<b>ORAL ADMINISTRATION</b>	Mixing with food, water, or treats.	Simple method for chronic dosing or substances absorbed through the GI tract.

	Using a gastric or nasogastric tube (gavage) directly into the stomach.	Ensures accurate dose administration.
<b>ADMINISTRATION BY INJECTION</b>	Intravenous (IV)	Directly into the vein; fastest effect; 100% bioavailability.
	Intramuscular (IM)	Into the muscle tissue.
	Subcutaneous (SC)	Under the skin (subcutaneous space).
	Intraperitoneal (IP)	Into the peritoneal cavity (abdomen).
<b>ADMINISTRATION BY INHALATION</b>	Using an inhalation chamber.	For volatile substances or aerosols.
	Using a mask.	Allows controlled delivery to the respiratory tract.
<b>TOPICAL ADMINISTRATION</b>	Applying creams, gels, or solutions directly to the skin.	For local effects or transdermal absorption.
	Applying to mucous membranes.	Targets local area (e.g., eye, nose).
<b>RECTAL ADMINISTRATION</b>	Using suppositories.	Bypasses first-pass metabolism; alternative when oral is not possible.
	Using enemas.	For local or systemic effects via the rectal mucosa.

#### **4.1 Choice of technique, volume and frequency of administration**

The choice of administration technique, volume, and frequency depends on several factors, including the substance's nature, the study's objective, the animal model used, and ethical constraints. Here are some considerations to guide these decisions:

##### **4.1.1 Nature of the Substance**

**Type of substance:** Some drugs or chemical compounds may require specific administration methods due to their physicochemical properties (stability, solubility or reactivity).

**Form of substance:** The substance can be in liquid, solid, or gaseous form, influencing the choice of administration method.

**Purpose of the administration:** P pharmacological study, toxicological study, treatment study, etc.

##### **4.1.2 The objective of the Study**

**Speed of action:** If a rapid response is required, intravenous administration is often preferred, allowing rapid diffusion into the bloodstream.

**Long-lasting effect:** Intramuscular, subcutaneous or transdermal administrations may be considered for prolonged release of the substance.

##### **4.1.3 Animal Model**

**Animal size:** The administration method should be adapted to the animal's size. For example, a feeding tube may be more appropriate for small rodents, while an intravenous injection may be more feasible for larger animals.

**Physiological characteristics:** The animal's physiology can influence the choice of method. Some animals tolerate specific techniques better than others.

#### **5. Ethical Constraints and Animal Welfare**

**Stress and pain:** Methods that minimise animal stress and pain should be preferred. For example, oral administration may be less invasive than some injection methods.

**Frequency of administrations:** If repeated administrations are necessary, less invasive methods may be preferred to minimise the impact on animal welfare.

### **5.1 Administration volume**

**Solubility of the substance:** The solubility in the chosen vehicle may limit the volume that can be administered.

**Physiological capacity of the animal:** Animals have limited physiological capacities to process a given volume, and excessive volumes can cause complications.

### **5.2 Frequency of Administration**

**Half-life of the substance:** The frequency of administration may depend on the substance's half-life in the body. Some medications must be administered frequently to maintain constant therapeutic levels.

**Experimental objective:** Some experimental protocols may require single administrations, while others may involve repeated administrations.

### **5.3 Specificities of Administration in Certain Animals**

**Intracerebral administration:** Used to target the brain directly.

**Orofecal administration:** Used to simulate natural ingestion conditions.

## **6. Dissection**

Dissection is a technique widely used in biology to study the internal structure of organisms. It involves methodically cutting out and examining the anatomical parts of an organism, often for educational, research, or medical purposes. The main steps of dissection are:

### **6.1 Equipment and Preparation**

**Material:** Scalpel, forceps, scissors, dissection trays, protective gloves, mask, safety glasses.

**Organisation:** A fresh or preserved specimen, often prepared specifically for dissection (animals, plants, organs, etc.).

### **6.2 Steps of dissection**

**Identification:** Identify external structures and their function before beginning dissection.

**Incision:** Make a precise incision in the area to be studied with a sharp scalpel.

**Exposure:** Use forceps or scissors to gently open the cavity or tissue layer and expose the internal organs.

**Observation:** Carefully examine the organs' position, shape, texture, and colour.

**Identification and Documentation:** Identify each structure and note its characteristics and location.

**Delicate handling:** Handle with care to avoid damage to internal structures.

### **6.3 Types of Dissection**

**Anatomical Dissection:** refers to the careful examination and separation of organs and tissues in an organism to study their structure, spatial relationships, and organization within the body. This technique provides fundamental insights into organ morphology, system architecture, and functional connectivity. Anatomical dissection is widely used in educational settings for teaching anatomy, in veterinary and medical research for understanding disease processes, and in experimental studies to evaluate organ-specific effects of treatments or interventions. During the procedure, organs are carefully exposed, preserved when necessary, and studied *in situ* or after removal, allowing for both qualitative observations and quantitative measurements, such as organ size, weight, and volume.

**Histological Dissection:** on the other hand, involves the microscopic examination of tissues after they have undergone specific processing steps, such as fixation, embedding, sectioning, and staining. This method allows researchers to visualize individual cells, cellular components, and tissue architecture, providing insights into cellular morphology, pathological changes, and molecular markers. Histological analysis is crucial for studying disease mechanisms, developmental biology, pharmacological effects, and tissue responses to interventions. Techniques such as Hematoxylin and Eosin (H&E) staining, immunohistochemistry, and fluorescent labeling enable detailed visualization of cell types, structural proteins, and biochemical pathways, making histological dissection a cornerstone of preclinical and translational research.

Together, anatomical and histological dissections complement each other: anatomical dissection provides a macro-level understanding of organ systems, while histological dissection offers a micro-level perspective, enabling researchers to correlate tissue structure with function and disease.

### **6.4 Ethical Considerations**

Ethical considerations are fundamental when performing anatomical or histological dissections to ensure that the welfare of animals is respected and that research or educational activities are conducted responsibly. A primary principle is respect for the animal, which entails using specimens that have been ethically sourced in accordance with institutional regulations, national laws, and

international standards. Animals should never be killed solely for dissection if alternative sources or models are available, and all procedures should follow established guidelines for humane treatment.

Another key principle is the responsible use of each specimen. Researchers and educators must strive to maximize the scientific or educational value obtained from every dissection, minimizing losses and waste. This includes careful planning of experiments, precise documentation, and efficient use of collected tissues for multiple analyses when possible. Ethical practices also extend to training personnel, ensuring that individuals performing dissections are skilled, knowledgeable about animal welfare, and aware of methods to reduce pain, stress, and unnecessary procedures.

By adhering to these ethical standards, researchers and educators not only protect animal welfare but also enhance the reliability, reproducibility, and societal acceptance of scientific work. Respectful and responsible handling of animal specimens demonstrates a commitment to high-quality research and education while maintaining moral and legal obligations.

### **6.5 Applications of Dissection**

Dissection serves as a fundamental tool in both education and scientific research, providing direct insights into organ structure, spatial relationships, and tissue organization that cannot be fully appreciated through textbooks or imaging alone. In an educational context, dissection is commonly employed to teach students about anatomy, organ systems, and physiological interconnections. It allows learners to gain hands-on experience, observe natural variations between specimens, and develop practical skills such as tissue handling, identification of structures, and understanding of complex organ relationships. This experiential learning fosters a deep comprehension of anatomy that is critical for medical, veterinary, and biological training.

In research, dissection is essential for studying specific organs, tissues, or disease processes. It enables the collection of high-quality samples for histological, molecular, biochemical, or functional analyses, allowing researchers to investigate pathophysiological mechanisms, effects of interventions, or organ-specific responses. Dissection also facilitates comparative anatomy studies, evaluation of developmental stages, and validation of animal models for human diseases. By providing direct access to organs and tissues, dissection remains a cornerstone of experimental

design, bridging the gap between observational data and mechanistic understanding in both basic and translational science.

### **6. 6 After the Dissection**

Following the completion of a dissection, it is essential to adhere to proper post-procedural practices to ensure safety, compliance, and the scientific value of the work. One critical aspect is the proper disposal of biological materials. All tissues, organs, and other biological waste must be handled and disposed of according to institutional regulations, national laws, and biohazard guidelines. This prevents contamination, environmental hazards, and the risk of accidental exposure to pathogens. Disposal methods may include incineration, autoclaving, or chemical treatment, depending on the type of material and local regulations.

Another key element is thorough documentation. Observations, measurements, and experimental outcomes should be accurately recorded in laboratory notebooks, databases, or digital systems. Detailed documentation ensures traceability, reproducibility, and transparency, enabling other researchers to interpret results, verify findings, and build upon the work. Recording any deviations, unexpected findings, or procedural difficulties is equally important, as these notes contribute to methodological rigor and continuous improvement in research or teaching practices.

By combining responsible disposal with meticulous documentation, researchers and educators uphold ethical standards, ensure safety, and maximize the scientific and educational value of each dissection, reinforcing both animal welfare principles and research integrity.

## **7. Techniques used for collecting physiological data**

Collecting physiological data during animal experiments is essential for understanding the effects of an intervention, drug, or other variables on the animals' bodies. Here are some of the techniques used to collect physiological data during animal experiments:

### **7.1 Cardiovascular monitoring**

**Electrocardiogram (ECG):** Record the electrical activity of the heart.

**Blood pressure measurement:** Using catheters or sensors placed in blood vessels.

**Blood flow:** By ultrasound, thermometry or specific measuring techniques.

### **7.2 Breathing**

**Spirometry:** Measurement of air volume and flow in the airways.

**Plethysmography:** Measurement of volume changes in the lungs or rib cage.

Body temperature:

**Thermometry:** Use of skin sensors or internal probes to measure body temperature.

### **7.3 Muscular and motor activity**

Electromyography (EMG): Record the electrical activity of muscles.

Movement analysis: Using motion sensors or cameras to study movement.

### **7.4 Neurology**

**Electroencephalography (EEG):** Recording of the brain's electrical activity.

**Brain imaging:** To study the brain, MRI (Magnetic Resonance Imaging) or PET (Positron Emission Tomography) is used.

### **7.5 Blood and biological analysis**

**Blood samples:** To measure levels of various substances such as hormones, inflammatory markers, etc.

**Biopsies and tissue analyses:** To study specific tissues and their responses to stimuli.

**Medical imaging:** Imaging techniques like magnetic resonance imaging (MRI) or computed tomography (CT) can obtain detailed images of internal organs and anatomical structures.

**Ultrasound: In real-time,** ultrasound uses sound waves to visualise internal organs, blood flow, and other anatomical structures.

## **8. Blood collection**

Blood collection in animal experiments is a standard procedure for obtaining biological samples to analyse various physiological parameters. This procedure must be carried out with the utmost care and comply with ethical and regulatory standards. The general steps for blood collection from laboratory animals are:

### **8.1 Method selection**

**By venipuncture:** Blood collection can be done from a vein, usually in the animal's neck, limbs, or tail.

**By cardiac puncture:** In some cases, collection can be done directly from the heart, but this requires advanced technical skills and is usually reserved for specific situations.

### **8.2 Anaesthesia or restraint**

During many experimental procedures, it is often necessary to anaesthetise the animal or maintain it under gentle restraint to minimize both stress and the risk of injury. Anaesthesia ensures that the animal remains immobile, unconscious, and free from pain, which is essential for invasive procedures, sample collection, imaging studies, or other manipulations that could otherwise cause discomfort or distress. The choice of anaesthetic, whether inhalant agents like isoflurane or sevoflurane, or injectable combinations such as ketamine-xylazine, depends on the species, procedure duration, and physiological considerations, including cardiovascular and respiratory effects.

When anaesthesia is not required, gentle physical restraint can be used to safely stabilize the animal during non-invasive procedures. Restraint techniques should be species-specific, minimally stressful, and designed to prevent injury to both the animal and the personnel. Common methods include padded restraining tubes, cloth wraps, or specialized handling devices, often combined with prior habituation or training to reduce anxiety and promote compliance.

Implementing anaesthesia or appropriate restraint not only serves ethical and welfare obligations but also enhances scientific validity. Stress and struggling can significantly alter physiological parameters, such as hormone levels, heart rate, immune responses, and metabolic activity, which may confound experimental results. By minimizing stress through these techniques, researchers ensure that the animal's physiological state remains as close to baseline as possible, improving the accuracy, reproducibility, and translational relevance of the collected data.

### **8.3 Preparation of the puncture site**

Before any invasive procedure involving puncture, such as blood sampling, injection, or catheter placement, the puncture area must be carefully prepared and disinfected to minimize the risk of infection and ensure the safety of the animal. The preparation process typically includes removing hair or fur around the site, either by clipping or trimming, followed by the application of antiseptic solutions such as 70% ethanol, iodine, or chlorhexidine.

Proper preparation serves multiple purposes. It reduces microbial contamination, preventing local or systemic infections that could compromise animal welfare and affect experimental outcomes. It also enhances visibility and accessibility of veins, arteries, or other anatomical structures, facilitating accurate and efficient puncture while minimizing trauma.

Personnel performing the procedure should follow strict aseptic techniques, including wearing gloves, using sterile instruments, and avoiding unnecessary contact with the prepared area. Maintaining sterility throughout the procedure is essential for ethical compliance, animal welfare, and the reliability of collected biological samples.

By meticulously preparing the puncture site, researchers reduce procedural complications, minimize pain or stress, and ensure that the data obtained are biologically valid and reproducible, supporting both scientific integrity and ethical standards in animal research.

#### **8.4 Needle selection**

Selecting an appropriate needle is a critical step in any procedure involving puncture, such as blood collection, injections, or intravenous catheterization. The size, gauge, and length of the needle must be carefully chosen based on the species, size, and age of the animal to minimize tissue trauma, reduce pain, and ensure efficient collection of samples or administration of substances.

Using a needle that is too large can cause excessive bleeding, hematoma formation, or unnecessary tissue damage, whereas a needle that is too small may impede sample collection, increase procedure time, and cause repeated attempts, thereby elevating stress and discomfort. For instance, small-gauge needles (27–30G) are typically used for mice and other small rodents, while larger needles (22–25G) are suitable for rats or larger species.

Proper needle selection also contributes to the accuracy and quality of the collected sample, which is essential for reliable biochemical, immunological, or molecular analyses. When combined with proper puncture site preparation and handling techniques, selecting the correct needle helps ensure that procedures are ethically compliant, minimally invasive, and scientifically valid, reducing variability caused by procedural complications.

#### **8.5 Blood collection:**

Once the needle is correctly positioned in the target vein or artery, blood can be collected into appropriately prepared tubes for subsequent analysis. The collection process must be performed carefully and efficiently to minimize stress, discomfort, and the risk of hemolysis, which could

compromise sample integrity. Tubes should be chosen based on the type of analysis planned, such as anticoagulant-coated tubes for plasma or serum separator tubes for serum collection.

During blood collection, it is important to monitor the animal continuously for signs of distress or excessive bleeding. The volume of blood drawn should be within safe limits relative to the animal's body weight, typically no more than 1% of total body weight per sampling session, to avoid hypovolemia or anemia. For repeated sampling, adequate recovery periods must be observed to ensure the animal's welfare and physiological stability.

Proper handling, combined with aseptic techniques and the use of appropriately sized needles, ensures that blood samples are of high quality, uncontaminated, and suitable for accurate biochemical, hematological, or molecular analyses. Meticulous attention to technique not only protects animal welfare but also enhances the reliability and reproducibility of experimental data, which is essential for translational research and valid scientific conclusions.

### **8.6 Sample processing**

Once blood samples have been collected, proper processing is essential to ensure their suitability for a variety of downstream analyses, including biochemical assays, hematological profiling, immunological studies, and the detection of specific biomarkers. The initial handling of samples should be rapid and precise, as delays or improper handling can lead to hemolysis, degradation of sensitive molecules, or changes in cellular and biochemical composition.

Depending on the intended analysis, blood may be centrifuged to separate plasma or serum, with careful removal of cellular components to prevent contamination. Anticoagulants, such as EDTA, heparin, or citrate, are used as appropriate to preserve the integrity of specific analytes or cell populations. For molecular studies, samples may be further processed to isolate DNA, RNA, or proteins, following standardized protocols to maintain quality and reproducibility.

Proper labeling, storage, and transport of samples are equally critical. Samples intended for immediate analysis should be kept at controlled temperatures, while those for long-term storage may require freezing at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  depending on the target analytes. Maintaining meticulous records of sample origin, collection time, and processing conditions ensures traceability and reproducibility, which are vital for reliable experimental results.

Overall, careful and standardized sample processing safeguards the accuracy, reliability, and translational relevance of the data obtained, while also adhering to ethical standards by maximizing the scientific value of each collected sample.

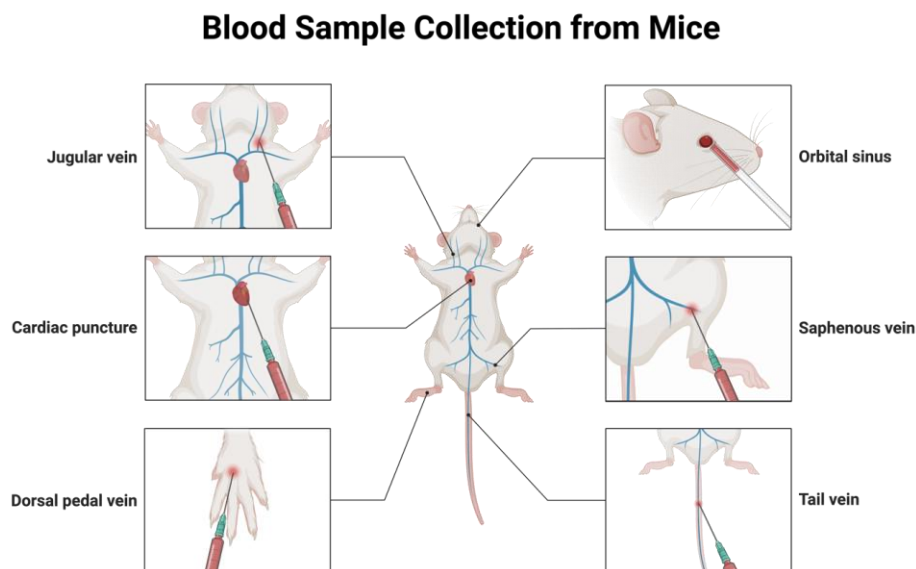
### **8.7 Post-collection care**

After blood or other sample collection, it is essential to provide the animal with appropriate post-procedural care to ensure recovery and minimize pain, stress, and potential complications. The collection site should be monitored for bleeding, hematoma formation, or signs of infection, and if necessary, gentle pressure or antiseptic application can be applied to aid hemostasis.

Animals should be returned to a comfortable, familiar environment with access to food, water, and social companions if applicable, as this helps reduce stress and promote rapid recovery. Monitoring should continue for a defined period post-procedure, checking for indicators of discomfort, lethargy, abnormal behavior, or physiological changes.

Pain management may include analgesics or anti-inflammatory agents, depending on the procedure and institutional guidelines. All interventions must comply with ethical standards and welfare regulations, ensuring that the animal's well-being is prioritized.

Providing attentive post-collection care not only protects the welfare of the animal but also enhances the quality and reliability of experimental data, as stress, pain, or poor recovery can alter physiological, biochemical, or behavioral parameters, potentially confounding study outcomes.



**Figure 19:** Blood sample Collection From mice

## **9. Urine collection**

Urine collection is commonly used in animal experiments to assess various physiological parameters. Here are some methods of urine collection in laboratory animals:

**Spontaneous collection:** Some animals can be trained to urinate on command in a special litter box or on a collecting surface. However, this method depends on the animal's cooperation and may not be appropriate for all types of cement.

**Manual stimulation:** Gentle abdominal or perineal stimulation can induce urine production in some animals, such as rats or mice. This can be done cautiously when collecting samples.

**Metabolic cages:** These special cages are designed to separate animals from their faeces and collect urine individually. This allows the amount of urine produced to be measured and its components analysed.

**Bladder catheterisation:** For more accurate sampling, a catheter can be inserted directly into the bladder through the urethra. However, this method is invasive and requires technical skill.

**Collection by puncture:** A fine needle can collect urine directly from the bladder by piercing the abdominal wall. This method is generally used when other methods are not feasible.

**Collection from surgically exposed bladder:** In some cases, surgery may be performed to expose the bladder, making it easier to collect urine.

## Urine Collection

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### Manual stimulation

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### Metabolic cages

These special cages are used to separate animals from their faeces and collect urine individually.



### Bladder catheterization

For more accurate sampling, a catheter can be inserted directly into the bladder.



### Collection by puncture

A fine needle can collect urine directly from the bladder by piercing the abdominal wall.



### Collection from surgically exposed bladder

In some cases, surgery may be performed to expose the bladder, making it easier



**Figure 20:** Urine Collection

## **10. Collection of fecal matter**

The collection of fecal matter is a standard and essential procedure in animal experiments, particularly in studies related to metabolism, nutrition, gastrointestinal health, and pharmacology. Fecal samples provide valuable information on nutrient absorption, gut microbial composition, metabolic by-products, and the effects of drugs, diets, or environmental interventions on the body. The methods used for fecal collection vary depending on the experimental design, duration of the study, and the species involved. For rodents, for example, animals can be placed in specialized metabolic cages that allow separation and collection of feces over a predetermined period. Alternatively, individual fecal pellets can be gently collected from the cage floor or directly from the animal with minimal handling to reduce stress. In larger animals such as rabbits, pigs, or non-human primates, feces can be collected using tray systems positioned under enclosures or during routine handling sessions, with careful timing to allow longitudinal monitoring of digestive or metabolic changes.

Once collected, fecal matter can be analyzed for a wide range of parameters. Nutrient composition analysis provides insight into protein, fat, fiber, vitamin, and mineral digestion and absorption. Hormonal metabolites such as corticosterone or leptin can be measured to assess stress, metabolic status, or reproductive function. The gut microbiome can be profiled using techniques such as 16S rRNA sequencing, metagenomics, or metabolomics to understand the diversity, composition, and functional activity of microbial communities. Fecal analysis also enables the study of drug metabolism and excretion, helping researchers understand how pharmaceuticals or dietary compounds are processed by the gastrointestinal system.

Ethical considerations are critical when collecting fecal samples. Procedures must be designed to minimize stress and discomfort to the animals, ensure proper welfare standards, and comply with institutional and regulatory guidelines. Proper handling, minimal restraint, and careful cage design not only protect animal welfare but also improve the reliability and reproducibility of experimental data, as stress and discomfort can alter metabolism, hormone levels, and microbial composition.



**Figure 21:** Collection of fecal matter

## **11. Organ and tissue donation**

Organ and tissue harvesting during animal experimentation is a common practice in biomedical research to study specific aspects of physiology and pathology or pathology and develop new medical treatments. These samples are generally collected for histological, biochemical, genetic, or molecular analysis.

## **12. Ethics and Regulations**

Complying with ethical and regulatory standards for animal research is essential. Researchers must obtain ethical approval from research ethics committees before undertaking organ removal experiments.

### **13. Anesthesia and Euthanasia**

Samples should be taken under anaesthesia to minimise pain and stress for the animal.

Euthanasia is often performed at the end of the experiment or after harvesting to avoid unnecessary suffering.

### **14. . Researcher Skills:**

Researchers must be trained and skilled in collection techniques to minimize complications and stress to the animal. This training not only enhances our competence but also ensures that we are equipped with the necessary skills to handle the animals with care and precision, making us feel skilled and competent.

- i. Minimising the Number of Animals Used** Researchers are encouraged to use the minimum number of animals necessary to obtain statistically significant results.
- ii. Use of Non-Invasive Techniques** Non-invasive techniques, such as medical imaging, may be preferred to avoid organ removal.
- iii. Post-Collection Considerations** Removed organs and tissues must be treated and preserved appropriately to maintain their integrity and allow for subsequent analysis.

### **15. Pain in animals during experimentation**

The issue of pain in animals used in experiments is crucial in terms of animal welfare and raises important ethical considerations. Pain can result from various experimental procedures, such as organ harvesting, injections, drug testing, etc.

#### **Key points to consider for pain management in experimental animals are:**

**Pain assessment and management:** Adequately assessing and managing pain in animals is essential. Species-specific pain assessment scales can be used to monitor and quantify pain.

Analgesics and anaesthetics are used daily to relieve p day. Trained professionals must administer these medications to ensure efficiency.

**Experimental planning** Experimental planning should incorporate methods to reduce or avoid pain as much as possible. Noninvasive methods may be preferred when possible.

**Post-procedural follow-up and care** Careful monitoring of animals after procedures is crucial to detect and manage any signs of persistent pain or post-operative complications.

**Ethics and regulations** Animal research regulations often require researchers to take steps to minimise pain and distress to animals used in their studies.

**Training and Awareness** Training researchers on recognising animal pain and best practices for managing it is essential.

**Reduction in Animal Use** One key approach is to promote alternative methods to animal testing whenever possible to reduce the number of animals subjected to painful procedures.

## **16. Euthanasia**

Euthanasia, the practice of humanely ending an animal's life in research, is a complex and sensitive issue. In the context of animal experimentation, euthanasia is often used at the end of studies or procedures to avoid unnecessary suffering and ensure respectful treatment of animals.

### **16.1 Reasons for euthanasia**

Euthanasia is performed to prevent **prolonged pain, suffering, or distress** in animals that can no longer be maintained in a humane condition or when their participation in an experimental protocol has ended. The decision to euthanize an animal is made following strict **ethical and scientific criteria**, ensuring that the procedure is both justified and conducted with respect for animal welfare.

From a scientific standpoint, euthanasia may be necessary when the animal reaches a **predetermined humane endpoint**, such as significant weight loss, tumor size, paralysis, or behavioral signs indicating irreversible suffering. These endpoints are established prior to the start of the experiment to minimize unnecessary pain and to comply with institutional and legal requirements for animal research.

In some cases, euthanasia is required **to obtain specific biological samples or tissues** that are critical for data analysis or post-mortem examination. Such procedures are carried out under approved protocols to ensure that the scientific benefits justify the termination of the animal's life.

Euthanasia may also be applied when **experimental objectives have been met** and continued maintenance of the animals would not contribute to additional scientific knowledge. Similarly,

animals that experience severe adverse effects, accidental injuries, or illness unrelated to the study are humanely euthanized to prevent suffering.

## 16.2 Methods of euthanasia:

Euthanasia methods must be **humane, reliable, and compatible with the species involved**, aiming to minimize pain, fear, and physiological distress. The chosen technique should lead to a **rapid loss of consciousness followed by cardiac and respiratory arrest**, ensuring that death occurs smoothly and irreversibly. Whenever possible, animals are first rendered unconscious through **anesthesia or sedation** to eliminate perception of pain before the final euthanasia step is performed.

### 16.2.1 Chemical Methods

Chemical methods are among the most commonly used due to their **predictability and minimal distress**.

- **Overdose of anesthetic agents** such as pentobarbital or isoflurane is considered the gold standard. These substances depress the central nervous system, causing loss of consciousness and respiration, followed by cardiac arrest.
- Inhalant anesthetics (e.g., halothane, sevoflurane, carbon dioxide under controlled conditions) can also be used, particularly for small animals, provided that exposure is gradual to reduce anxiety.
- In some cases, **intravenous or intraperitoneal injection** of approved euthanasia solutions is preferred for precise dosage control.

### 16.2.2 Physical Methods

Physical methods are employed when chemical agents are impractical or unavailable and must be performed by trained personnel to ensure **instantaneous insensibility**.

- Examples include **cervical dislocation** (for small rodents and birds), **decapitation**, and **penetrating captive bolt** (for larger animals such as pigs or cattle).
- These methods must be executed swiftly and precisely to avoid prolonged distress, and are often preceded by anesthesia to ensure complete unconsciousness.

### 16.2.3 Confirmation of Death

Following any euthanasia procedure, it is essential to **verify the death** of the animal by confirming **absence of heartbeat, respiration, and reflexes**. Failure to ensure complete death can cause unnecessary suffering and is considered ethically unacceptable. In some protocols, a **secondary method** (such as exsanguination or bilateral thoracotomy) is applied as confirmation.

### 16.2.4 Ethical and Legal Oversight

All euthanasia methods must conform to **institutional animal care guidelines** and **international standards**, such as those established by the **American Veterinary Medical Association (AVMA)**, the **European Directive 2010/63/EU**, or equivalent national regulations. The procedure should be documented in detail, including the method used, personnel involved, and justification for the chosen approach.

## 17. Ethical and regulatory considerations:

Euthanasia protocols must comply with established regulations and ethical standards for animal research. Researchers must be trained to perform euthanasia competently and compassionately.

**Post-euthanasia care** Particular attention must be paid to the management of animals after euthanasia, including the respectful disposal of bodies.

**Development of human criteria** Researchers and ethics committees are working to develop criteria that determine the appropriate time to perform euthanasia while respecting animal welfare.

**Thinking about alternatives** It is important to continually consider alternatives to the use of animals and experimental methods that minimise the need for euthanasia.

## CHAPTER 8: NOTION OF THE ANIMAL MODEL

### 1. Introduction

The concept of animal models plays a central role in scientific research because it provides information on human diseases and potential therapies. Animal models are chosen based on their phylogenetic, physiological, and anatomical similarities to humans and are classified as exploratory, explanatory, and predictive models based on their utilisation in research. The development of transgenic models has further improved the relevance of animal models by allowing precise genetic modifications to be introduced, facilitating the study of disease mechanisms in animals that do not naturally exhibit the conditions. However, animal models are not limited, and alternative methods, such as cellular models, are being explored to address ethical concerns and enhance research outcomes.

### 2. Choice of the Animal Model in Scientific Research

Animal models are selected based on their genetic and anatomical similarities to humans [Sreelatha *et al.*, 2024].

They are classified as naive or induced models, with naive models encompassing spontaneously occurring disease models and induced models created using various techniques, including CRISPR-Cas9 [Sreelatha *et al.*, 2024].

The choice of the animal model is crucial for the validity of research results, as it influences the capacity to control variables and conduct genetic tests [O'Neil *et al.*, 1999].

#### 2.1 Relevance for Human Diseases

Animal models should closely mimic human diseases to ensure translational relevance. Rodents like rats and mice are commonly used due to their physiological similarities with humans and the availability of genetically modified models [S *et al.*, 2024].

Zebrafish have gained importance for their genetic tractability and rapid development, making them suitable for studying neurodegenerative and metabolic disorders [Madivalar *et al.*, 2024; Patel & Srinivasan, 2024]. Animal models should closely mimic human diseases to ensure translational relevance. Rodents such as rats and mice are commonly used due to their physiological

similarities with humans and the availability of genetically modified models [S et al., 2024]. Zebrafish have gained significance for their genetic tractability and rapid development, making them suitable for studying neurodegenerative and metabolic disorders [Madivalar et al., 2024; Patel & Srinivasan, 2024].

**Table 4: Common Animal Models and Their Applications**

<i>Animal Model</i>	<i>Key Advantages</i>	<i>Examples of Research Applications</i>
<i>Mice</i>	Genetic similarity to humans, well-characterized genome, ease of manipulation	Cancer research, immunology, neurodegenerative diseases, drug testing
<i>Rats</i>	Larger size than mice, suitable for physiological studies	Cardiovascular research, toxicology, behavioral studies
<i>Zebrafish</i>	Rapid development, optical transparency, genetic tractability	Developmental biology, drug screening, neurodegenerative disorders

## **2.2 Ethical Considerations**

Ethical concerns related to animal welfare necessitate adherence to the 3Rs principles: replacement, reduction, and refinement [Neziri *et al.*, 2024].

Researchers are encouraged to explore alternative methods, such as *in vitro* techniques, to minimize the use of animals while obtaining reliable data [Neziri *et al.*, 2024].

## **2.3 Specificity of Disease Models**

The complexity of diseases, such as those affecting the cardiovascular system, necessitates a rigorous selection of models capable of reproducing essential pathophysiological and comorbidity features [Parikh & Pierce, 2024].

**Table 5 : Examples of Disease Models and Key Features Modeled**

<i>Disease Area</i>	<i>Animal Model Examples</i>	<i>Key Features Modeled</i>
<i>Cardiovascular Disease</i>	Mouse models of atherosclerosis, rat models of hypertension	Plaque formation, blood pressure dysregulation, cardiac dysfunction
<i>Neurodegenerative Disorders</i>	Mouse models of Alzheimer's, zebrafish models of Parkinson's	Amyloid-beta plaques, tau tangles, motor deficits, neuronal loss
<i>Metabolic Disorders</i>	Mouse models of diabetes, zebrafish models of obesity	Insulin resistance, hyperglycemia, weight gain, lipid metabolism abnormalities

### 3. Physiological and Behavioral Assessment in Animal Models

#### 3.1 Evaluation of Physiological Parameters

Accurate physiological assessment is critical for ensuring that animal models faithfully replicate human disease mechanisms. Researchers routinely monitor vital signs, metabolic parameters, hormonal levels, immune responses, and organ-specific biomarkers to validate the relevance of a given model. These measurements not only confirm the presence of disease phenotypes but also provide quantitative endpoints for testing therapeutic interventions.

For example, in rodent models of Alzheimer's disease, monitoring neuroinflammatory markers such as IL-1 $\beta$ , TNF- $\alpha$ , and microglial activation helps establish mechanistic parallels with human pathology, supporting translational relevance. Similarly, in models of cardiovascular disease, continuous measurement of heart rate, blood pressure, and ECG patterns provides crucial information about cardiac function and responses to pharmacological treatments.

Advances in technology have greatly enhanced the accuracy, reproducibility, and non-invasiveness of physiological monitoring:

- **Telemetry systems** allow wireless, real-time recording of heart rate, ECG, blood pressure, and body temperature without restraining animals.

- **Metabolic cages** enable continuous measurement of food and water intake, energy expenditure, glucose metabolism, and respiratory parameters.
- **Imaging techniques** such as MRI, PET, and CT provide **high-resolution structural and functional data**, allowing longitudinal studies of organ morphology, neural activity, or tumor progression.
- **Biosensors and wearable devices** facilitate minimally invasive monitoring of blood glucose, oxygen saturation, and other biomarkers in awake animals, reducing stress-induced artifacts.

**Table 6: Key Physiological Parameters, Methods, and Applications in Animal Models**

Physiological Parameter	Measurement Method	Animal Model Example	Scientific/Clinical Application
Heart Rate & ECG	Telemetry, ECG leads	Rodents, rabbits, dogs	<b>Cardiovascular research</b> , arrhythmia studies, drug safety screening
Blood Pressure	Tail-cuff, telemetry, catheterization	Rats, mice, rabbits	<b>Hypertension studies</b> , cardiac function evaluation, hemodynamics
Glucose & Metabolic Markers	Blood sampling, continuous glucose monitors (CGM), metabolic cages	Rodent models of diabetes/obesity	<b>Diabetes research</b> , metabolic disorder assessment, efficacy of anti-diabetic drugs
Hormonal Levels	ELISA, radioimmunoassay (RIA), multiplex assays	Rodents, primates	<b>Endocrinology studies</b> , stress response evaluation, reproductive research
Immune Function	Flow cytometry, ELISPOT, cytokine assays	Mice, rats	<b>Autoimmune disease models</b> , vaccine testing, assessment of immunotherapies
Neuroinflammation Markers	Immunohistochemistry (IHC), ELISA, PET imaging	Alzheimer's, Parkinson's models	<b>Neurodegeneration studies</b> , neuroinflammatory response evaluation, drug target validation

Organ Morphology & Function	MRI, PET, CT, ultrasound	Mice, rats, non-human primates (NHP)	<b>Oncology</b> , cardiology, neurobiology, tissue regeneration assessment
Respiratory Function	Plethysmography, blood gas analysis	Rodents, larger mammals	<b>Pulmonary disease models</b> (e.g., asthma, COPD), toxicology studies, anesthetic safety
Activity & Energy Expenditure	Metabolic cages, telemetry	Rodent obesity/metabolism models	<b>Obesity</b> , metabolic syndrome, <b>circadian rhythm studies</b> , caloric restriction effects

### 3.2 Behavioral and Cognitive Testing

Behavioral assessments in animal models are essential for understanding neurological, cognitive, and psychological processes that cannot be fully captured through anatomical or molecular analyses alone. These tests provide quantitative and qualitative measures of an animal's cognitive function, emotional state, motor abilities, and social behavior, which are critical for modeling human neurological and psychiatric disorders.

Commonly used paradigms include:

- **Morris Water Maze:** Evaluates spatial learning and memory by assessing the ability of rodents to locate a hidden platform in a water-filled pool, reflecting hippocampal-dependent cognitive function.
- **Open-Field Test:** Measures general locomotor activity, exploration, and anxiety-like behaviors by recording movement patterns, rearing, and time spent in the center versus the periphery of an arena.
- **Rotarod Test:** Assesses motor coordination, balance, and motor learning by recording the time an animal can remain on a rotating rod, which is particularly useful for studying neurodegenerative disorders affecting the motor system.
- **Elevated Plus Maze:** Evaluates anxiety-like behavior based on the animal's willingness to explore open versus enclosed arms in a raised maze, providing insight into emotional regulation and stress response.

These behavioral indices are crucial for studying disorders such as Parkinson's disease, Alzheimer's disease, depression, anxiety, and autism spectrum disorders, enabling researchers to correlate molecular and cellular alterations with functional outcomes. Furthermore, combining multiple behavioral paradigms allows for a comprehensive phenotypic profile, which enhances the translational relevance of preclinical studies and improves the predictive power of therapeutic interventions.

Advanced approaches, such as automated video tracking, machine learning-based behavior recognition, and longitudinal monitoring, are increasingly integrated into behavioral research. These technologies increase the accuracy, reproducibility, and objectivity of behavioral measurements, while also reducing human bias and stress induced by handling.

#### **4. Standardization, Reproducibility, and Experimental Design**

##### **4.1 Importance of Standardization**

Variations in housing conditions, diet, light cycles, and handling can significantly influence experimental results. Therefore, the standardization of animal husbandry practices is essential to reduce variability.

Organizations such as the Federation of European Laboratory Animal Science Associations (FELASA) and AAALAC International provide guidelines to harmonize animal research conditions globally. Proper documentation of environmental factors improves data comparability and scientific transparency.

##### **4.2 Statistical Power and Reproducibility**

Reproducibility crises in biomedical research often arise from poor experimental design and inadequate sample sizes. Using appropriate statistical power calculations ensures that experiments involve the minimum number of animals necessary to obtain valid results.

Additionally, randomization, blinding, and preregistration of protocols are critical practices that minimize bias and enhance the credibility of animal studies. Many journals and funding agencies now require strict adherence to these principles before publication.

## **5. Translational Value and Integration with Human Data**

### **5.1 Bridging Preclinical and Clinical Research**

A major goal of animal modeling is to ensure translational validity, that is, how the extent to which findings from animals can be applied to humans. Integrating multi-omics data (genomic, proteomic, metabolomic) from both animal and human studies helps identify shared biomarkers and pathways.

This integrative strategy increases confidence that discoveries made in animal models will hold true in clinical trials, reducing costly failures in drug development.

### **5.2 The Role of Artificial Intelligence and Computational Modeling**

Recent advances in machine learning and systems biology allow researchers to analyze complex datasets generated from animal models. AI algorithms can predict disease progression, optimize dosing strategies, and identify the most predictive models for specific human disorders.

By combining biological experimentation with computational modelling, modern research is transitioning toward a data-driven, precision-based paradigm, in which animal and digital models complement each other to facilitate faster, more ethical, and more reliable biomedical discovery.

## **6. Animal Welfare and Experimental Management**

### **6.1 Monitoring and Husbandry Practices**

Animal welfare constitutes a fundamental pillar of ethical and responsible scientific research. Ensuring the well-being of laboratory animals is not only an ethical obligation but also directly impacts the reliability and validity of experimental results. Stress, discomfort, or poor living conditions can profoundly alter physiological, behavioral, and immunological responses, leading to confounding effects that compromise data quality.

Proper environmental enrichment is essential to minimize stress and promote natural behaviors. Environmental parameters such as lighting, temperature, humidity, noise levels, and air quality must be carefully controlled to meet species-specific requirements. For example, rodents and lagomorphs are sensitive to sudden changes in light or temperature, which can influence circadian rhythms, metabolic activity, and stress hormone levels. Providing appropriate bedding, nesting material, and shelters allows animals to exhibit natural behaviors such as burrowing, hiding, and thermoregulation. Social species should have opportunities for controlled social interaction, while maintaining separation when necessary to prevent aggression or injury. Environmental enrichment

also includes toys, tunnels, and climbing structures for physical activity and mental stimulation, which help reduce stereotypic behaviors and enhance overall well-being.

Routine veterinary supervision is critical to detect early signs of pain, distress, or disease. Daily health checks should monitor parameters such as body weight, grooming habits, feeding and drinking behavior, posture, and activity levels, as these are reliable indicators of physiological and psychological well-being. Any deviations from normal patterns may indicate illness, stress, or discomfort, prompting timely intervention. Veterinary oversight also ensures that procedures, housing, and enrichment strategies adhere to the latest standards of animal care and welfare.

Humane handling techniques are equally important. Proper training of personnel in gentle handling, restraint, and transfer procedures minimizes stress, reduces the risk of injury, and improves the accuracy of behavioral and physiological measurements. Handling should be consistent, predictable, and adapted to the species, age, and health status of the animals.

The refinement of housing and care practices aligns with international guidelines and regulatory frameworks, such as those established by the Federation of European Laboratory Animal Science Associations (FELASA) and the Guide for the Care and Use of Laboratory Animals. These standards emphasize species-appropriate housing, environmental enrichment, monitoring, veterinary care, and ethical review of experimental protocols. Implementation of these guidelines ensures that research institutions not only comply with legal requirements but also uphold the highest ethical standards, fostering a culture of respect for animal life and welfare.

Overall, the integration of proper environmental enrichment, veterinary supervision, daily health assessment, and humane handling is critical to maintaining animal welfare. Such practices reduce stress, prevent disease, enhance natural behavior, and ultimately contribute to robust, reproducible, and ethically sound scientific research. By prioritizing animal well-being, researchers uphold ethical responsibility while improving the quality and reliability of experimental outcomes.

## **6.2 Training and Competence of Personnel**

The quality, reliability, and ethical integrity of animal research are highly dependent on the training and competence of personnel involved in experimental procedures. Researchers, technicians, and animal care staff must possess a thorough understanding of animal biology, behavior, and welfare, as well as practical skills in the handling, anesthesia, and monitoring of laboratory animals. Competent personnel are essential for recognizing signs of pain, distress, or illness, allowing timely interventions that minimize suffering and prevent confounding experimental results.

Personnel must be proficient in performing anesthesia and analgesia, ensuring that procedures are conducted safely and humanely, with minimal stress to the animals. They should also be trained in euthanasia methods and the application of humane endpoints, which are predetermined criteria that dictate when an animal should be removed from a study to prevent unnecessary pain or suffering. Understanding and implementing these techniques requires both theoretical knowledge and hands-on experience under qualified supervision.

To maintain high standards, institutions often implement continuous education programs, certification courses, and practical workshops that cover species-specific care, procedural techniques, ethical principles, and regulatory requirements. Supervision by Institutional Animal Care and Use Committees (IACUCs) or equivalent oversight bodies ensures compliance with local, national, and international guidelines. These committees provide guidance on protocol design, monitor training effectiveness, and verify that personnel consistently adhere to welfare standards.

Well-trained personnel contribute directly to animal welfare, scientific validity, and experimental reproducibility. Proper handling reduces stress-induced physiological changes, while accurate application of techniques ensures consistency across experiments. Furthermore, skilled staff can detect subtle behavioral or physiological indicators of discomfort that might otherwise go unnoticed, enabling early interventions that protect both the animals and the integrity of the data.

## **7. Innovation and Future Directions in Animal Modeling**

### **7.1 Integration of Multispecies and Multi-Scale Models**

Modern biomedical research increasingly relies on a comparative and integrative approach that leverages the strengths of multiple animal species and experimental systems to generate a more comprehensive understanding of biological processes. This strategy, often referred to as multi-species and multi-scale modeling, enables researchers to capture complementary insights that cannot be obtained from a single species or experimental level alone.

Rodent models, such as mice and rats, are widely used due to their genetic manipulability, well-characterized physiology, and rapid breeding cycles. They provide detailed molecular- and cellular-level data, allowing the study of gene function, signaling pathways, and disease mechanisms. Zebrafish, in contrast, offer advantages in developmental biology, as their rapid embryogenesis, optical transparency, and amenability to high-throughput imaging allow researchers to observe organ development, vascularization, and neural circuitry in real time. Non-human primates are

employed when higher-order cognitive functions, complex immune responses, or social behaviors are of interest, as their physiology and neurobiology more closely resemble humans.

By integrating data across these species, researchers can triangulate findings, identify conserved biological mechanisms, and validate experimental results across multiple levels of complexity. This approach enhances the translational relevance of preclinical research, increasing the likelihood that discoveries in animal models will predict human responses.

In addition to in vivo models, multi-scale frameworks often incorporate in vitro organoid systems, tissue cultures, and computational simulations. Organoids allow the study of cellular interactions, tissue architecture, and drug responses in a controlled environment, complementing in vivo observations. Computational models further enable predictive simulations, network analysis, and integration of large datasets, providing insights into system-level dynamics that may be difficult or impossible to study directly in living animals.

The integration of multispecies and multi-scale approaches also addresses ethical considerations by promoting the 3R principles—replacement, reduction, and refinement. By combining data from several models, researchers can reduce the number of animals required, replace certain in vivo studies with organoid or computational systems, and refine experimental protocols to minimize suffering while still achieving meaningful scientific outcomes.

Overall, this integrative strategy strengthens scientific rigor, reproducibility, and translational potential, creating a more complete picture of complex biological phenomena. It exemplifies the modern trend in biomedical research toward systems-level understanding, bridging the gap between molecular mechanisms and whole-organism physiology while balancing scientific innovation with ethical responsibility.

## **7.2 Next-Generation Technologies in Model Development**

Emerging technologies are reshaping the landscape of preclinical science.

- **CRISPR multiplex editing** enables simultaneous modification of several genes, creating complex polygenic disease models.
- **Single-cell omics** and **spatial transcriptomics** allow detailed mapping of cellular responses in animal tissues.
- **3D bioprinting** and **human-animal chimeric systems** are being explored to replicate specific organ environments with unprecedented accuracy.

These innovations represent a paradigm shift toward **personalised and precision modeling**, enhancing the predictive power of animal studies while maintaining strict ethical oversight.

**Table 7: Next-Generation Technologies for Preclinical Models**

<b>Emerging Technology</b>	<b>Description/Functionality</b>	<b>Application in Model Development</b>	<b>Key Impact and Advantages</b>
<b>CRISPR Multi-plex Editing</b>	Enables the simultaneous modification of several genes within a single organism or model.	Creation of complex polygenic disease models (involving multiple genes).	More accurate simulation of the genetic complexity of human diseases, enhancing the relevance of animal models.
<b>Single-Cell Omics and Spatial Transcriptomics</b>	Detailed mapping and analysis of cellular responses (gene expression, proteins, etc.) in animal tissues, at single-cell resolution and/or by preserving their spatial location.	Allows for detailed mapping of tissues and specific cellular responses in animal models.	Provides in-depth understanding of cellular heterogeneity and disease mechanisms, increasing data resolution.
<b>3D Bioprinting and Human-Animal Chimeric Systems</b>	Use of 3D printing to create in vitro tissue or organ structures, and integration of human cells/tissues into animal systems (chimeras).	Highly accurate replication of specific organ environments (Organ-on-a-Chip, Organoids) for disease and drug studies.	Improves the predictability of in vivo/in vitro studies by more closely matching human physiology, while maintaining ethics (reducing animal use).

## **8. End-of-Experiment Procedures and Ethical Termination**

### **8.1 Humane Euthanasia and Endpoint Determination**

Euthanasia is performed to prevent prolonged suffering or distress in animals when their participation in research is complete or humane endpoints are reached. Humane endpoints are predefined criteria based on clinical signs, weight loss, behavioral changes, tumor size, or irreversible organ dysfunction.

The selection of euthanasia methods must be species-specific, rapid, and minimally distressing, following international guidelines such as the AVMA Guidelines for the Euthanasia of Animals and institutional protocols. Common methods include overdose of anesthetic agents, inhalant anesthesia, and, where appropriate, physical methods such as cervical dislocation or decapitation, always ensuring prior anesthesia when necessary.

### **8.2 Post-Euthanasia Handling and Tissue Collection**

After euthanasia, animals may be used for tissue collection, histological analysis, or molecular studies. Procedures must be performed rapidly and ethically to preserve sample integrity while respecting welfare principles.

Proper documentation of euthanasia procedures, tissues collected, and timing ensures transparency, reproducibility, and ethical accountability.

In some cases, carcass disposal is managed according to biosafety regulations, including incineration, burial, or rendering, ensuring environmental safety and compliance with local laws.

## **9. Data Integration, Reporting, and Societal Impact**

### **9.1 Transparent Reporting and Reproducibility**

Accurate and transparent reporting of animal experiments is essential for scientific integrity and reproducibility. Researchers are encouraged to follow guidelines such as ARRIVE (Animal Research: Reporting of In Vivo Experiments), which cover experimental design, randomization, blinding, sample size, and ethical considerations.

Clear reporting allows other researchers to replicate findings, validate results, and avoid unnecessary duplication of animal experiments, aligning with the 3Rs principle.

## **9.2 Contribution to Science and Public Health**

Animal models remain a cornerstone of biomedical research, providing indispensable insights into the mechanisms of human physiology, disease progression, and therapeutic interventions. Their use enables researchers to explore complex biological processes in a controlled environment, which is often not feasible in humans due to ethical or practical constraints. By carefully designing studies with appropriate species and experimental models, scientists can investigate pathophysiological pathways, genetic contributions, immune responses, and organ-specific effects, all of which inform the development of effective medical treatments.

One of the most visible contributions of animal research is in the development of vaccines, drugs, and clinical therapies. For instance, animal studies are crucial in preclinical testing, determining safety, efficacy, pharmacokinetics, and potential side effects before human trials. These studies have led to breakthroughs in infectious disease control, oncology, cardiovascular medicine, neurodegenerative disorders, and immunology. Animal models also provide valuable data for understanding rare diseases or conditions with complex genetic and environmental interactions, offering a translational bridge from molecular discoveries to clinical application.

Beyond experimental insights, the ethical and responsible use of animals reinforces public confidence in scientific research. Adhering to stringent welfare standards, including the 3Rs (Replacement, Reduction, and Refinement), ensures that animal studies are conducted only when necessary and with minimal suffering. Transparent reporting, ethical review processes, and regulatory oversight communicate to the public that research institutions prioritize both scientific integrity and humane treatment. This trust is essential for maintaining societal support for research initiatives and for facilitating the translation of preclinical findings into human health benefits.

Emerging trends in research further enhance the societal impact of animal models. Open data initiatives and collaborative networks allow researchers to share findings across institutions, reducing redundant experiments and increasing reproducibility. Integration with human clinical data, such as patient-derived samples, epidemiological datasets, and longitudinal health records, creates a multi-level understanding of disease, bridging preclinical and clinical research. This approach not only accelerates the translation of findings into evidence-based medical practices but also informs public health policies, guides regulatory decisions, and supports the development of preventive strategies and healthcare interventions.

Overall, animal models contribute significantly to scientific advancement and public health improvement, offering mechanistic understanding, preclinical validation, and translational pathways that ultimately benefit human and animal populations alike. The combination of ethical responsibility, rigorous methodology, and integrative research approaches ensures that animal experimentation remains a valuable, socially responsible, and scientifically impactful component of modern biomedical research.

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