Name of Unit	Unit :1
Subject /Course name	Experimental Pharmacology
Subject/Course ID	BP810ET
Class: B.Pharm. Semester	VIII
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Learning Outcome of Module 01

LO	Learning Outcome	Course Outcome Code
LO 01	To remember and understand the CPCSEA and OECD guidelines for the maintenance, breeding, and experimental use of laboratory animals.	BP810.1
LO 02	To understand and explain the characteristics and applications of common laboratory animals, including different species, strains, transgenic, and mutant animals.	BP810.1
LO 03	To apply appropriate techniques for drug administration, blood collection, and euthanasia in laboratory animals.	BP810.1
LO 04	To analyze the importance of ethical handling and proper techniques in laboratory animal experimentation.	BP810.1

Content Table

Topic
• Laboratory Animals • Study of CPCSEA and OECD guidelines for maintenance, breeding and conduct of experiments on laboratory animals. Common Lab Animals • Description and applications of different species and strains of animals. • Popular transgenic and mutant animals. • Techniques for Collection of Blood • Common routes of drug administration in laboratory animals. • Techniques of blood collection and euthanasia.

MODULE:01

INTRODUCTION TO PHARMACOLOGICAL SCREENING METHODS

Experimental pharmacologist after considering the existing knowledge of the aetiology and pathophysiology of the diseases with the clinically-active drugs of known pharmacological properties, developed models that can mimic pathophysiological conditions in animals. These animal models would help to discover and screen the newer agents for clinical trial. Thus, the methods or models that are employed to screen the activity of newly identified agents (test compound/substance) especially for understanding its pharmacological actions and its therapeutic potential are called as pharmacological screening methods.

Screening of drugs involves two things; one is a test or group of tests believed to permit the detection of physiological activity of drugs (Scanning) and second is to reduce the uncertainty of the scanning (Evaluation). The pharmacological screening comprises following objectives:

- To get pharmacological activity of chemically unidentified substances.
- To investigate the function of endogenous mediators in the action of test substance.
- To find out the toxicity or unwanted action of test substances.

The above pharmacological screening objectives are achieved on the basis of pre-clinical trial. The pre-clinical trial or non-clinical trial (1-2 years) are laboratory tests for new chemicals or drugs or agents or medical devices, which are generally performed on the animal models or bacterial culture to see whether the treatment really work and are tested on animals to ascertain the safety and efficacy of agents or medical devices before considering it for human use. Thus, during the evaluation process, unfavorable compound can be rejected via a process of pre-clinical trials and only few agents pass for testing on humans.

The test compound undergoes several steps during pre-clinical trials to ensure its clinical use, which are as follows:

1. File for approval through an investigational new drug (IND) application for ethical clearance via CPCSEA.

2. Establishing an effective and toxic dose of the test drug by following OECD guidelines.

3. Screen the drug for its pharmacological activity.

4. Developing a bio-assay for test drug.

5. Identify the drug target of action (receptor, ion channel, enzyme etc. in a body).

In other words, the pre-clinical trials include many studies to ascertain the following aspects of test drugs:

1. To indicate the presence or absence of pharmacodynamic activity of drugs for example, analgesic activity, anti-diabetics etc.

2. To detect specific activity of test substance on isolated preparation or bacterial culture (such as an anti-histaminic, vasodilator, anti-bacterial etc.).

3. To elucidate the mechanism of action (to find out the mechanism of action for example whether the anti-hypertensive effect of a test drug is due to α-blocker or β-blocker or calcium channel blocker or ACE inhibitors activity).

4. To understand the systemic pharmacology of test drug (effect on other major organ or system like nervous system, cardiovascular system, renal system, gastric system, respiratory system etc. apart from clinical target).

5. The purpose is to conduct pharmacokinetic studies and quantitative tests in order to establish the dose–response relationship, determine the maximum effect, and assess comparative efficacy and bioavailability of different formulations.

6. The objective is to determine the toxicity profile of the test substance by performing acute and chronic toxicity studies.

The schematic representation of the pharmacological screening process (preclinical development of a new test drug/compound/substance)

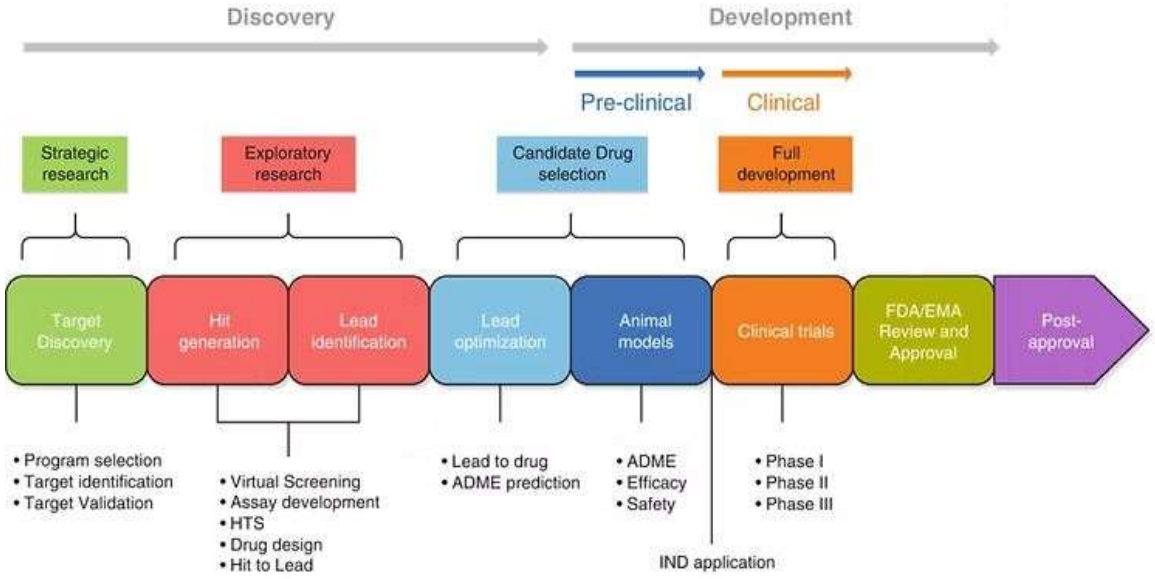


Fig1.1 Preclinical and Clinical stages of Drug Screening

TYPES OF PHARMACOLOGICAL SCREENING TECHNIQUES

Pharmacological screening is performed to establish the pharmacologic activity of new unidentified compound, to identify its endogenous mediators, identify the toxic and unwanted effect of compound. However, depending on the amount of test substance extracted or synthesized the pharmacological screening is performed on animal models using in vitro, in vivo or in silico techniques.

1. ***In vitro techniques (outside body):***

The effect of test drug is studied on the isolated tissues or organs of the body in control condition. For example activity assay (screen the activity), bioassay using specific isolated target tissue or organs (to define the molecular mechanism, determination of potency of chemical or biological agents like hormones, ions and drugs), toxicity assay laboratory-based assays performed outside a living organism, including chemical assays (anti-oxidant assay, DNA, RNA, protein assay) and cell culture study (immunological assay, cancer cell line, receptor binding studies).

2. ***In vivo techniques (inside body):***

In this type of screening, the test drugs are directly administered in the body using laboratory animals like rodent such as rat, mice, etc., or large animals like cat, dog or monkey etc., and effect on overall system or specific organ function is noted. Moreover, ex-vivo experimental protocols are also performed outside the living body in an artificial environment that may last for 2-3 h.

3. ***In silico techniques (computer aided drug designing):***

Considering the ethical concern for the use and number of laboratory animals in pharmacological screen of test substance, in silico studies can also be performed before the animal studies using computer aided models or via computer simulator to predict the activity of the test with computer simulated targets like enzyme, receptor binding efficacy. This type of approach will help to reduce the number of animals utilized initially during the screening of various newly synthesized derivatives in different doses. Such type of screening is called as computer aided drug designing or screening.

TYPES OF PHARMACOLOGICAL SCREENING METHODS

Depending upon the history or knowledge of the origin of the test compound and probable information available about the pharmacology of the test drug, the pharmacological screening of it can be performed using following screening methods:

- ✓ Simple Screening
- ✓ Blind Screening
- ✓ Programmed Screening

(I) Simple Screening: In simple screening one or two tests are performed, when there is little knowledge about the compound. For example: a single test for the concentration of sugar in blood may be used to screen for hypoglycaemic activity of compounds.

Merits:
(a) Inexpensive and less time consuming.
(b) No need for battery or series of tests.

Demerits:
(a) Only applicable for substances that are active in single way.
(b) Selected only for suitable well defined methods.
(c) Accuracy in result is not achieved.

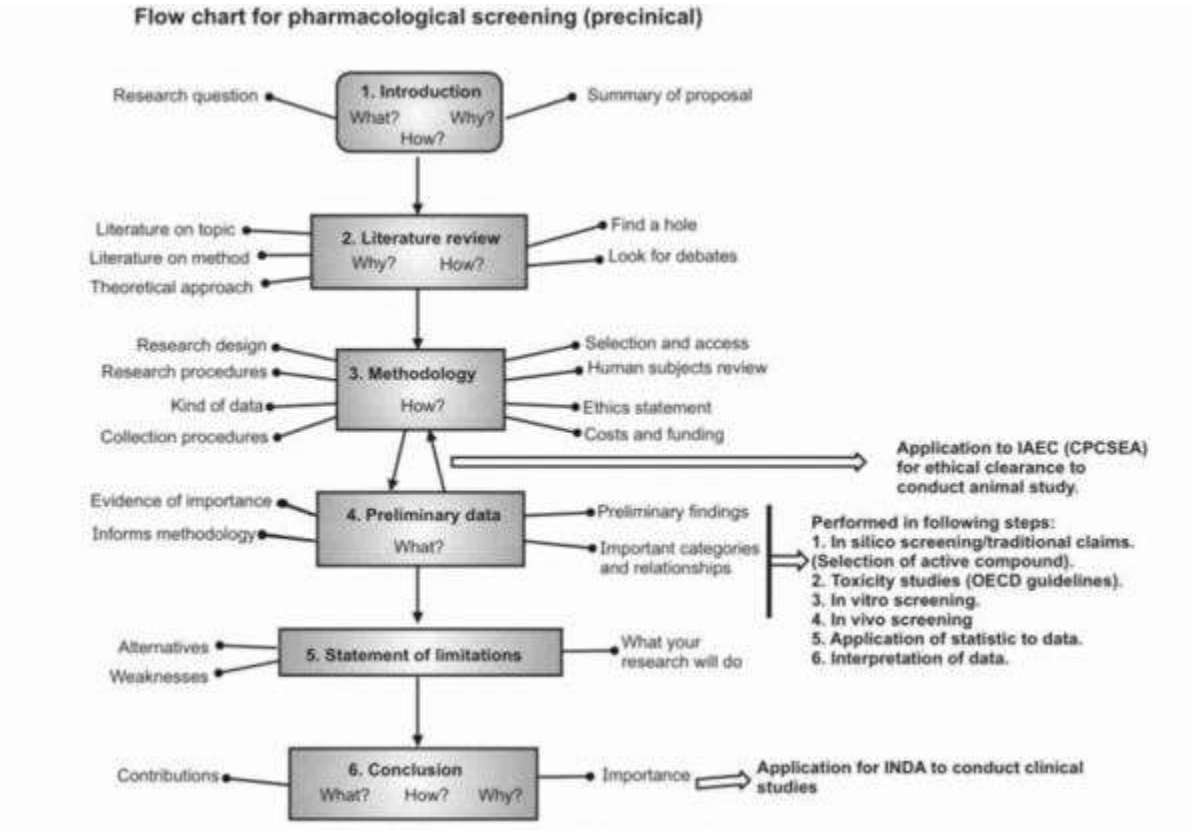
(II) Blind Screening: The blind screening is used only when nothing is known or there is no information about the pharmacological activity of the test compound. In such case, one has to perform many tests and toxicological pathway is essential for every compound.

Merits:
(a) To demonstrate whether new group of substances is worthy for further attention.
(b) Point out the most potent chemical with interesting pharmacological activity.

Demerits:
(a) Require planning and skilful execution of all the tests.

(III) Programmed Screening: Programmed screening is performed when activity of one compound is known and other activities are unknown. Such screening provides information about how the compounds are active in what ways. This method also provides information about the main activity and subsidiary activity of the compound. Moreover, due to some knowledge about the parent compound, the potency of the test compound can be compared with known compound. Thus, it is easy for experimental pharmacologist to decide whether to proceed or terminate the further screening of the test compound. However, limited data about the test compound is obtained than blind screening. A series of testing programs are required to provide information on the compounds or targets.

The general steps to be followed during te screening of new drugs are :



CPCSEA /CCSEA

The motto of Prevention of Cruelty to Animals (PCA) Act 1960 as amended in 1982, is to prevent infliction of unnecessary pain or suffering on animals. The Central Government as enumerated under PCA Act 1960 has constituted a Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) which is duty bound to take all such measures as may be necessary to ensure that animals are not subjected to unnecessary pain or suffering before, during or after the performance of experiments on them. For this purpose, the Government has made "Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998" as amended during 2001 and 2006, to regulate the experimentation on animals.

The objective of this SOP is to contribute to the effective functioning of the Institutional Animals Ethics Committee (IAEC) so that a quality and consistent ethical review mechanism for research on animals is put in place for all proposals dealt by the Committee as prescribed by the CPCSEA under PCA Act 1960 and Breeding of and Experimentation (Control and Supervision) Rules 1998, as amended in 2001 and 2006.

IAEC has been designed to secure the following objectives:

(a) Every experiment shall be performed by or under the supervision of a person duly qualified in that behalf, that is, Degree holders in Veterinary Science or Medicine or Laboratory Animal Science of a University or an Institution recognised by the Government for the purpose and under the responsibility

of the person performing the experiment;

(b) That experiments are performed with due care and humanity and as far as possible experiments involving operations are performed under the influence of some anaesthetic of sufficient power to prevent the animals from feeling pain

(c) That animals who, in the course of experiments under the influence of anaesthetics, are so injured that their recovery would involve serious suffering, are ordinarily medically allowed to death while still under influence of anaesthetic

(d) That experiments on animals are avoided wherever it is possible to do so.

(e) That experiments on larger animals are avoided when it is possible to achieve the same results by experiments on small laboratory animals like guinea-pigs, rabbits, mice, rats etc

(f) That, as far as possible, experiments are not performed merely for the purpose of acquiring manual skill

(g) That animals intended for the performance of experiments are properly looked after before, during and after experiments;

(h) That suitable records are maintained with respect to experiments performed on animals

Composition of IAEC Institutional Animals Ethics committee

It shall include eight members as follows:

A. IAEC members from the establishment (05 members):

- ✓ One biological scientist
- ✓ Two scientists from different biological disciplines
- ✓ One veterinarian involved in the care of animal
- ✓ One scientist in charge of animal facility of the establishment concerned
- ✓ The Chairperson of the Committee and Member Secretary would be nominated by the establishment from amongst the above five members. However, if the establishment wants to propose its administrative head, who is from non-scientific background, as Chairperson, then six members of IAEC may be proposed.

Having a Veterinarian in IAEC is mandatory for judging level of care and handling of Laboratory animals in a given protocol.

B. Nominees from the CPCSEA:

- ✓ Main Nominee (01)
- ✓ Link Nominee
- ✓ Scientist from outside the Institute (01)
- ✓ Socially Aware Nominee (01)

The CPCSEA shall endeavour that nominees appointed by CPCSEA in any private establishment should not be from the establishments which is having the same objectives as to the establishment where the nominees are being nominated, so as to avoid the conflict of interest between the establishments.

CPCSEA GUIDELINES FOR LABORATORY ANIMAL FACILITY

Good Laboratory Practices (GLP) for animal facilities is intended to assure quality maintenance and safety of animals used in laboratory studies while conducting biomedical and behavioral research and

testing of products.

GOAL

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioral research and testing with the basic objective of providing specifications that will enhance animal well-being, quality in the pursuit of advancement of biological knowledge tl1at is relevant to humans and animals.

VETERINARY CARE

Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience 'in laboratory animal sciences and medicine. Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behaviour, and well-being is conveyed to the attending veterinarian. The veterinarian can also contribute to the establishment of appropriate policies and procedures for ancillary aspects of veterinary care, such as reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs; and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

ANIMAL PROCUREMENT

All animals must be acquired lawfully as per the CPCSEA guidelines. A health surveillance program for screening incoming animals should be carried out to assess animal quality. Methods of transportation should also be taken into account (Annexure - 4). Each consignment of animals should be inspected for compliance with procurement specifications, and the animals should be quarantined and stabilized according to procedures appropriate for the species and circumstances.

QUARANTINE, STABILIZATION AND SEPARATION

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. A minimum duration of quarantine for small lab animals is one week and larger animals is 6 weeks (cat, . dog, monkey, etc.) Effective quarantine procedures should be used for non-human primates to help limit exposure of humans to zoonotic infections. Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychologic and nutritional stabilization before their use. The length of time stabilization will depend on the type and duration of animal transportation, the species involved and the intended use of the animals. Physical separation of animals by species is recommended to prevent interspecies disease transmission and to eliminate anxiety and possible physiological and behavioral changes due to interspecies conflict. Such separation is usually accomplished by housing different species in separate rooms; however, cubicles, laminar-flow units, cages that have filtered air or separate ventilation, and isolators shall be suitable alternatives. In some instances, it shall be acceptable to house different species in the same room, for example, if two species have a similar pathogen status and are behaviorally compatible.

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

All animals should be observed for signs of illness, injury, or abnormal behavior by animal house staff. As a rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that-appropriate methods be in place for disease surveillance and diagnosis (Annexure 1 & 2). Unexpected deaths and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious agent (e.g. Mycobacterium tuberculosis in non-human primates), the group should be kept intact and isolated during the process of diagnosis, treatment, and control. Diagnostic clinical laboratory may be made available.

ANIMAL CARE AND TECHNICAL PERSONNEL

Animal care programs require technical and husbandry support. Institutions should employ people trained in laboratory animal science Or provide for both formal and onthe-job training to ensure effective implementation of the program

PERSONAL HYGIENE

It is essential that the animal care staff maintain a high standard of personal cleanliness. Facilities and supplies for meeting this obligation should be provided e.g. showers, change of uniforms, footwears etc. Clothing suitable for use in the animal facility should be supplied and laundered by the institution. . A commercial laundering service is acceptable in many situations; however, institutional facilities should be used to decontaminate clothing exposed to potentially hazardous microbial agents or toxic substances. In some circumstances, it is acceptable to use disposable gear such as gloves, masks, head covers, coats, coveralls and shoe covers. Personal should change clothing as often as is necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility. Washing and showering facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics in animal rooms. A separate area or room should be made available for these purposes. .

ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

Institutions should have policies governing experimentation with hazardous agents. Institutional Bio safety Committee whose members are knowledgeable about hazardous agents are in place in most of the higher level education, research institutes and in many. pharmaceutical industries for safety issues. This committee shali also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions

Since the use of animals in such studies requires special consideration, the procedures and the facilities to be used must be reviewed by both the Institutiona_ Biosafety committee and Institutional Animal Ethics Committe'e

MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

Multiple surgical procedures on a single animal for any testing or experiment are not to be practiced unless specified in a protocol only approved by the IAEC.

DURATIONS OF EXPERIMENTS

No animal should be used for experimentation for more than 3 years unless adequate justification is provided.

PHYSICAL RESTRAINT

Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal. Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to research objectives. Less restrictive systems, such as the tether system or the pole and collar system, should be used when compatible with research objectives. The following are important quidelines for the use of restraint equipments: Restraint devices cannot be used simply as a convenience in handling or managing animals. The period of restraint should be the minimum required to accomplish the research objectives. Animals to be placed in restraint devices should be given training to adapt to the equipment. Provision should be made for observation of the animal at appropriate intervals. Veterinary care should be provided if lesions or illness associated with restraint are observed. The presence of lesions, illness, or severe behavioral change should be dealt with by the temporary or permanent removal of the animal from restraint.

PHYSICAL PLANT

The physical condition and design of animal facility determine, toa great, extent, the efficiency and economy of their operation. The design and size of an animal facility depend on the scope of institutional research activities, animals to be housed, physical relationship to the rest of the institution, and geographic location. A well-planned, properly maintained facility is an important element in good animal care. .

PHYSICAL RELATIONSHIP OF ANIMAL FACILITIES TO LABORATORIES

Good animal husbandry and human comfort and health protection require separation of animal facilities from personnel areas such as offices, conference rooms, and most laboratories. ÿ Laboratory animals are very sensitive to their living conditions. It is important that they shall be housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds. The building, cages and environment of animal rooms are the major factors, which effect the quality of animals.

This separation can be accomplished by having the animal quarters in a separate building, wing, floor, or room. Careful planning should make it possible to place animal housing areas adjacent to or near laboratories, but separated from them by barriers such as entry locks, corridors, or floors. ÿ In planning an animal facility the space should be well divided for various activities. The animal rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilized for services such as stores, washing, office and staff, machine rooms, quarantine and corridors. The environment of animal room (Macro-Environment) and animal cage (Micro-Environment) are factors on which the production and experimental efficiency of the animal depends. Since animals are very sensitive to environmental. changes, sharp fluctuations in temperature, humidity, light, sound and ventilation should be avoided. The recommended space requirements for animal rooms, for different species are given in

FUNCTIONAL AREAS

The size and nature of a facility will determine whether areas for separate service functions are possible or necessary. Sufficient animal area required to: ÿ Ensure separation of species or isolation of individual projects when necessary;

Receive, quarantine, and isolate animals; and Provide for animal housing. In facilities that are small, maintain few animals or maintain animals under special conditions (e.g., facilities exclusively used for housing germfree colonies or animals in runs and pens some functional areas listed below could be unnecessary or included in a multipurpose area.

Professional judgement must be exercised when developing a practical system for animal care.

- Specialized laboratories or Individual areas contiguous with or near animal housing areas for such activities as surgery, intensive care, necropsy, radiography, preparation of special diets, experimental manipulation, treatment, and diagnostic laboratory procedures containment facilities or Equipment, if hazardous biological, physical, or chemical agents are to be used
- Receiving and storage areas for food, bedding
- Pharmaceuticals and biologics, and supplies
- Space for administration, supervision, and direction of the facility
- Showers, sinks, lockers and toilets for personnel
- An area for washing and sterilization equipment and supplies
- An autoclave for equipment ÿ Food, and bedding; and separate areas
- For holding soiled and cleaned equipment
- An area for repairing cages and equipment
- An area to store wastes prior to incineration or removal

PHYSICAL FACILITIES

(a) Building materials should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

(b) Corridor(s) should be wide enough to facilitate the movement of personnel as well as equipments and should be kept clean.

(c) Utilities such as water lines drain pipes, and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.

ANIMAL ROOM DOORS

Doors should be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.

EXTERIOR WINDOW

Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate source of light and ventilation. In primate rooms, windows can be provided.

FLOORS

Floors should be smooth, moisture proof, nonabsorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants. They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints. A continuous moisture-proof membrane might be needed. If sills are installed at the entrance to a room, they should be designed to allow for convenient passage of equipment.

DRAINS

Floor drains are not essential in all rooms used exclusively for housing rodents. Floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Where floor drains are used, the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying of surfaces.

WALLS & CEILINGS

Walls should be. free of cracks, unsealed utility penetrations, or .imperfect junctions with doors, ceilings, floors and corners. Surface materials should be capable of withstanding scrubbing with detergents ad disinfectants and the impact of water under high pressure.

STORAGE AREAS

Separate storage areas should be designed for feed, bedding, cages and materials not in use. Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal tissue waste.

FACILITIES FOR SANITIZING EQUIPMENT AND SUPPLIES

An area for sanitizing cages. and ancillary equipment is essential with adequate water supply

EXPERIMENTAL AREA

All experimental procedures in small animals should be carried out in a separate area away from the place where animals. are housed. For larger animal functional areas for aseptic surgery should include a separate surgical support area, a preparation area, the operating room or rooms, and an area for intensive care and supportive treatment of animals.

ENVIRONMENT

(a) TEMPERATURE AND HUMIDITY CONTROL

Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions or differences. in the number and kind of room occupants. Ideally, capability should be provided to allow variations within the range of approximately 18 to 29°C (64.4 to 84.2fF), which includes the temperature ranges usually recommended for common laboratory animals. The relative humidity should be controllable within the range of 30% to 70% throughout the year. For larger animals a comfortable zone (18 to 37°C) should be maintained during extreme summer by appropriate methods for cooling.

VENTILATION

In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals' primary enclosures. Heating, ventilating, and air-conditioning systems should be designed so that operation can be continued with a standby system. The animal facility and human

occupancy areas should be ventilated separately.

(c) POWER AND LIGHTING

The electrical system should be safe and provide appropriate lighting and a sufficient number of power outlets. It suggested that a lighting system be installed that provides adequate illumination while people are working in the animal rooms and a lowered intensity of light for the animals. Fluorescent lights are efficient and available in a variety of acceptable fixtures. A time-controlled lighting system should be used to ensure a regular diurnal lighting cycle wherever required. Emergency power should be available in the event of power failure.

(d) NOISE CONTROL

The facility should be provided with noise free environment. Noise control is an important consideration in designing an animal facility. Concrete walls are more effective than metal or plaster walls containing noise because their density reduces sound transmission.

ANIMAL HUSBANDRY

(a) CAGING OR HOUSING SYSTEM The caging or housing system is one of the most important elements in the physical and social environment of research animals. It should be designed carefully to facilitate animal well being, meet research requirements, and minimize experimental variables. The housing system should:

- Provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species; (Annexure - 3)
- Provide a comfortable environment
- Provide an escape proof enclosure that confines animal safety
- Provide easy access to food and water
- Provide adequate ventilation
- Meet the biological needs of the animals, e.g., maintenance of body temperature, urination, defecation, and reproduction.

Keep the animals dry and clean, consistent with species requirements; Facilitate research while maintaining good health of the animals.

They should be constructed of sturdy, durable materials and designed to minimize crossinfection between adjoining units. Polypropylene. polycarbonate and stainless steel cages should be used to house small lab animals, Monkeys should be housed in cages made of steel or painted mild steel and for other animals such as sheep, horses, the details can be seen in Annexure - 3. To simplify servicing and sanitation, cages should have smooth, impervious surfaces that neither attract nor retain dirt and a minimum number of ledges, angles, and comers in which dirt or water can accumulate. The design should allow inspection of cage occupants without disturbing them. Feeding and watering devices should be easily accessible for filling, changing, cleaning and servicing. Cages, runs and pens must be kept in good condition to prevent injuries to animals, promote physical comfort, and facilitate sanitation and servicing. Particular attention must be given to eliminate sharp edges and broken wires, keeping cage floors in good condition.

FOOD

Animals should be fed palatable, non-contaminated, and nutritionally adequate food daily unless the

experimental protocol requires otherwise. Feeders should allow easy access to food, while avoiding contamination by urine and feces. Food should be available in a mounts sufficient to ensure normal growth in immature animals and maintenance of normal body weight, reproduction, and lactation in adults. . Food should contain adequate nutrition, including formulation and preparation; freedom from chemical and microbial contaminants; bioavailability of nutrients should be at par with the nutritional requirement of the animal. . Laboratory animal diets should not be manufactured or stored in facilities used for farm feeds or any products containing additives such as rodenticides, insecticides, hormones, antibiotics, fumigants, or other potential toxicants. Areas in which diets are processed or stored should be kept clean and enclosed to prevent entry of insects or other animals.

Precautions should be taken if perishable items such as meats, fruits, and vegetables are fed, because these are potential sources of biological and chemical contamination and can also lead to variation in the amount of nutrients consumed. Diet should be free from heavy metals (e.g., lead, arsenic, cadmium, nickel, mercury), naturally occurring toxins and other contaminants. Exposure to extremes in relative humidity, unsanitary conditions, light, oxygen, and insects hasten the deterioration of food. Meats, fruits, vegetables, and other perishable items should be refrigerated if required to be stored. Unused, open food should be stored in vermin - proof condition to minimize contamination and to avoid potential spread of disease agents. Food hoppers should not be transferred from room to room unless cleaned and sanitized. The animal feed should contain moisture, crude fibre, crude protein, essential vitamins, minerals crude fat and carbohydrate for providing appropriate nutrition.

BEDDING

Bedding should be absorbent, free of toxic chemicals or other substances that could injure animals or personnel, and of a type not readily eaten by animals. Bedding should be used in amounts sufficient to keep animals dry between cage changes without coming into contact with watering tubes. Bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry. The frequency is a matter of professional judgement of the animal care personnel in consultation with the investigation depending on the number of animals and size of cages. However it is ideal to change the bedding twice a week. The desirable criteria for rodent contact bedding is ammonia binding, sterillizable, deleterious products not formed as a result of ste.rilization, easily stored, non desiccating to the animal, uncontaminated, unlikely to be chewed or mouthed, non - toxic, non - malodorous, nestable, disposable by incineration, readily available, remains chemically stable during use, manifests batch uniformity, optimizes normal animal behaviour, non - deleterious to cage - washers, non injurious and non - hazardous to personnel, non - nutritious and non - palatable. Nesting materials for newly delivered pups wherever can be provided (e.g. Paper, tissue paper, cotton etc.).

OECD Guidelines

A unique tool for assessing the potential effects of chemicals on human health and the environment. Internationally accepted standard methods for safety testing. It is used by professionals in industry, academia, and government for testing and assessing chemicals (industrial chemicals, pesticides, personal care products, etc.).Regularly updated with input from thousands of national experts from OECD member countries. It is covered by the Mutual Acceptance of Data (MAD), meaning data generated in an OECD member or partner country following OECD Test Guidelines and Good Laboratory Practice (GLP) are accepted in other OECD countries for assessment and protection of human health and the environment.

Hundreds of new chemicals (industrial chemicals, pesticides, food additives, biotechnology products, and pharmaceuticals) enter the global market each year, requiring safety testing worldwide.

Purpose of the guidelines:

- ✓ Enhance the validity and international acceptance of test data.
- ✓ Make the best use of available resources in governments and industry.
- ✓ Avoid the unnecessary use of laboratory animals.
- ✓ Minimise non-tariff trade barriers.

These are OECD Guidelines for the Testing of Chemicals, covering various toxicity tests and study types.

Acute Toxicity

- ✓ Oral Toxicity (401, 420, 423, 425)
- ✓ Dermal Toxicity (402)
- ✓ Inhalation Toxicity (403, 436)
- ✓ Eye Irritation/Corrosion (405, 437, 438, 491, 492)
- ✓ Dermal Irritation/Corrosion (404, 430, 435)

Repeated Dose Toxicity

- ✓ 28-day Oral Toxicity (407)
- ✓ 90-day Oral Toxicity (408, 409)
- ✓ Dermal Toxicity (410, 411)
- ✓ Inhalation Toxicity (412, 413)

Reproductive & Developmental Toxicity

- ✓ Prenatal Development (414)
- ✓ Reproduction Toxicity (415, 416, 421, 422, 443)
- ✓ Developmental Neurotoxicity (426)

Genetic Toxicity & Carcinogenicity

- ✓ Bacterial Reverse Mutation (471)
- ✓ Mammalian Cell Tests (473, 476, 487, 490)
- ✓ In Vivo Tests (474, 475, 478, 483, 484, 485, 488, 489)
- ✓ Carcinogenicity (451, 452, 453)

Neurotoxicity

- ✓ Organophosphorus Substances (418, 419)
- ✓ Neurotoxicity Study (424, 426)

Skin & Eye Effects

- ✓ Skin Sensitization (406, 429, 442A, 442B, 442C, 442D)
- ✓ Skin Absorption (427, 428)
- ✓ Eye Effects (437, 438, 460, 491, 492)

Endocrine Disruptors

- ✓ Estrogen Receptor Assays (455, 440, 441, 455, 457, 493)
- ✓ Steroidogenesis Assay (456)

1. Acute Toxicity

1. Oral Toxicity (OECD 401, 420, 423, 425)
These tests determine the harmful effects of a chemical after a single oral dose. They help estimate the LD_{50} (lethal dose) and classify chemicals according to toxicity.

2. Dermal Toxicity (OECD 402)
This test evaluates the toxic effects of a chemical when applied to the skin. It measures systemic toxicity and helps determine safe exposure levels.

3. Inhalation Toxicity (OECD 403, 436)
These studies assess the toxic effects of chemicals when inhaled as gases, vapors, or aerosols. They determine the LC_{50} and evaluate respiratory toxicity.

4. Eye Irritation/Corrosion (OECD 405, 437, 438, 491, 492)
These tests determine whether a substance causes eye irritation or serious eye damage. Both animal and alternative in-vitro methods are used.

5. Dermal Irritation/Corrosion (OECD 404, 430, 435)
These studies evaluate whether chemicals cause skin irritation or corrosion after topical application. They help classify substances for skin safety.

2. Repeated Dose Toxicity

1. 28-day Oral Toxicity (OECD 407)
This study evaluates the effects of repeated daily oral exposure for 28 days. It helps identify target organs and establish safe exposure levels.

2. 90-day Oral Toxicity (OECD 408, 409)
This sub-chronic study examines toxic effects of continuous oral exposure for 90 days. It provides information on cumulative toxicity and dose–response relationships.

3. Dermal Toxicity (OECD 410, 411)
These tests evaluate toxic effects after repeated skin exposure over several weeks. They help assess long-term dermal safety.

4. Inhalation Toxicity (OECD 412, 413)
These studies assess the effects of repeated inhalation exposure to chemicals over short or sub-chronic periods. They evaluate respiratory and systemic toxicity.

3. Reproductive & Developmental Toxicity

1. Prenatal Development (OECD 414)
This test evaluates the effect of chemicals on pregnant females and fetal development. It identifies developmental toxicity and possible birth defects.

2. Reproduction Toxicity (OECD 415, 416, 421, 422, 443)
These studies assess the impact of chemicals on fertility, reproductive performance, and offspring development across one or more generations.

3. Developmental Neurotoxicity (OECD 426)
This study examines whether exposure to chemicals during development affects the nervous system and brain function of offspring.

4. Genetic Toxicity & Carcinogenicity

1. Bacterial Reverse Mutation Test (OECD 471)
Also known as the Ames test, it detects whether chemicals cause gene mutations in bacteria, indicating potential mutagenic risk.

2. Mammalian Cell Tests (OECD 473, 476, 487, 490)
These in-vitro tests evaluate chromosomal damage, gene mutations, or DNA damage in cultured mammalian cells.

3. In Vivo Tests (OECD 474, 475, 478, 483, 484, 485, 488, 489)
These tests detect genetic damage in living animals, confirming mutagenic effects observed in vitro.

4. Carcinogenicity (OECD 451, 452, 453)
Long-term studies that evaluate whether prolonged exposure to a chemical causes cancer or tumor formation in animals.

5. Neurotoxicity

1. Organophosphorus Substances (OECD 418, 419)
These tests assess delayed neurotoxicity caused by organophosphorus compounds, especially their effects on the nervous system.

2. Neurotoxicity Study (OECD 424, 426)
These studies evaluate functional and structural effects on the nervous system following chemical exposure.

6. Skin & Eye Effects

1. Skin Sensitization (OECD 406, 429, 442A–442D)
These tests determine whether a chemical can cause allergic skin reactions after repeated exposure.

2. Skin Absorption (OECD 427, 428)
These studies measure the rate and extent of chemical absorption through the skin.

3. Eye Effects (OECD 437, 438, 460, 491, 492)
These tests evaluate eye irritation, corrosion, or damage, often using alternative in-vitro methods.

7. Endocrine Disruptors

1. Estrogen Receptor Assays (OECD 440, 441, 455, 457, 493)
These assays detect chemicals that interfere with estrogen hormone signaling, indicating potential endocrine disruption.

2. Steroidogenesis Assay (OECD 456)
This test evaluates whether chemicals affect hormone synthesis, particularly steroid hormones such as testosterone and estrogen.

ANIMALS USED IN EXPERIMENTAL PHARMACOLOGY

Animal experimentation is a term used to describe the use of animals in study, training, and education. Cutting into or dissecting a live animal is known as vivisection and it is a concept preferred by those who reject the use of animals in research. It has been reported that animals are exposed to stressful educational and training practices that are unnecessary. Concerns have been posed on how animals are killed in these "irrelevant tests" due to the extensive use of animals in toxicity research and the testing of dermatological preparations." CPCSEA aims to ensure that animals are not subjected to unnecessary suffering or pains during, before, or after the experiments on them. In India, a large number of animals are used in various experiments and studies. Animals have been used and are still approved for drug testing, bioassay screening, and preclinical testing, including general and detailed studies of toxicity. This preclinical safety and efficacy data must be submitted to the drug regulatory authorities before permission to conduct further human trials can be granted. In pure research, a greater variety and a

larger number of animals are used than in applied research. Examples include embryogenesis, developmental genetics, behavior, and breeding experiments in fruit flies, nematodes, mice, and rats. In the pharmaceutical industry or universities, applied research aimed at addressing specific questions is normally carried out. The University Grants Commission (UGC), CPCSEA, and Medical Council of India (MCI) all recommend three Rs in animal experiments: replacement, reduction, and refinement, with a fourth R, rehabilitation, added as an added step for their treatment . The creation of alternatives is a necessity in today's changing scenario. In several medical colleges across India, using live animal experiments is reducing. These are increasingly being replaced by those alternatives that are available with demonstrated educational effectiveness and at reasonably low cost.

Different Species are used in Experimental Study

1**. Rat** : Albino rats are one of the commonest laboratory animals because of their small size and greater sensitivity to most drugs. There is a wide head, rough fur, and long ears on the albino rat. The length of the tail is often less than the length of the body. It has a long cylindrical body, a long thin tail, and legs that are very short.

The head has 2 silt-like nostrils with a pointed snout, a narrow mouth with a split upper lip and short lower jaw, 2 tiny beady eyes, set so that they can look forward and sideways diagonally, and they have several long whiskers. It has a short neck. The trunk is slightly wider than the head. Rats do not vomit because they lack a vomiting center. They do not have a gall bladder.

Experimental Use: Analgesic and anticonvulsant studies, bioassays of various hormones such as insulin, oxytocin, and vasopressin, chronic blood pressure studies, gastric acid secretion studies, acute and chronic toxicity studies The rats are ideal for determining the teratogenicity and carcinogenicity of drugs. Tissues of Rat: Tissues of rats used for various drug actions.

Blood Collection: By snipping the tip of the tail, small blood samples can be obtained from a tail vein, large amounts of blood can be collected from anesthetized rats by heart puncture or by orbital sinus.

2. **GUINEA PIG** : For decades, the guinea pig has been utilized as a laboratory animal in human experimental subjects, and it is a docile animal. The guinea pig is a short, tail-free rodent, with small ears, the head is seen in the profile is rectangular and blunt. The thick short, neck of the trunk emerges. The length of the limbs is unequal, while the forelegs are shorter than the hind legs. In many respects, the guinea pig differs from other laboratory rodents. It needs Vitamin C in the diet and is very susceptible to anaphylactic shock and tuberculosis. They are highly sensitive to histamine. Guinea pig is usually utilized as a metaphor for a scientific experimentation subject. They have been used to separate various bacterial strains in the past, but they have been replaced by mice and rats in modem laboratories, which replicate faster.

Experimental Use: It is mainly used for the evaluation of the bronchodilator drugs against experimentally induced asthma (histamine or acetylcholine aerosols). It is also used in the field of immunology particularly in the area of delayed hypersensitivity by using various antigens such as egg albumin, horse serum, etc. They have widely used in the studies of the local anesthetics as well as the bioassay of digitalis and suitable for hearing experiments as they have sensitive cochlea and experiments on oxygen consumption. It resembles a man in that it also needs an exogenous source of vitamin C and so it is useful in the study of its metabolism. Being an appropriate host for Mycobacterium, it is also suitable for tuberculosis studies. Various Isolated Tissues of Guinea Pig: Isolated organ preparations such as guinea pig lungs and intestines are widely used. These organ and tissue preparations were considerable in the discovery and early development of medicines to treat stomach ulcers and also beta-blockers to treat high blood pressure. The terminal ileum is most sensitive for the preliminary screening of the spasmodic and antispasmodic compounds and suitable for the detection and assay of histamine and related compounds.

Blood Collection: Small blood samples (less than 0.25 ml) can be collected by simple venesection of the marginal ear vein; large amounts of an anesthetized guinea pig by cardiac puncture or by metatarsal veins; repeated samples of small quantities can be collected from the orbital sinus.

3. **MOUSE (Mus Musculus)** Albino mice are the smallest laboratory animals, which can be bred uniformly. They have a smooth hair coat and a slim body with a long-pointed snout, prominent round ears, and long flat front teeth. They are cheap and easy to handle.

Experimental Use: Mice are widely useful in acute toxicity studies. They are also used in the assay of insulin and analgesics and also for the general screening of the chemotherapeutic agents specially bred mice arc mainly useful in the study of problems in genetics and cancer. They are most frequently used for testing drugs due to teratogenicity. The Nude mice which lack the thymus gland are mainly useful in the study of tissue immunity and transplantation research.

Tissues of Mice: Tissues of mice are used in the vas deferens and the ileum is the only tissue used in mice for the experiments because it is small and delicate.

4. **RABBIT** Rabbits are docile animals. The rabbit's body, except in a few areas (the tip of the nose, a small part of the scrotum, and the inguinal spaces), is tightly coated with smooth hair or fur. With readily visualized musculature, the rabbit has an erect and large pinna (external ears). An undivided lower lip and a cleated upper lip are connected by the tiny external opening of the mouth. The external nostrils are ovoid and in the upper lip are attached to the cleft. Around the nose, over each eye, and one or two on each cheek, prominent whiskers are present. The rabbit has wide eyes that are more laterally focused (pink in albino rabbit) than most mammals. The hind legs are longer than the forelimbs, muscular and strong. It has a huge caecum and a long appendix. In rabbits, the gene for atropinesterase is linked with the color of the fur. The enzyme atropine esterase is present in rabbit plasma and liver, so it can tolerate a large dose of belladonna.

Experimental Use: Rabbits are mainly used for pyrogen testing in intravenous fluids. The agents affecting capillary permeability are primarily examined by intracutaneous injection of the substances and accompanied by intravenous dye injection, such as Evans blue. Studies of miotics and mydriatics, Insulin, and other antidiabetic drugs, curare, and sex hormones are tested in the rabbit. It is used in serological work and for the screening of embryo toxic agents. It is mainly used for the research on reproduction as ovulation is nonspontaneous and its semen is also collected easily. The convenience of injecting into and withdrawing blood from the ear marginal vein is helpful in bioavailability studies. Used Tissues of Rabbit: Isolated heart, Jejunum, and ileum are some of the preparations routinely used for the testing of the drugs. Blood Collection: The marginal ear vein is the chosen blood collection site; blood may also be withdrawn from the jugular vein, orbital sinus, for significant volumes (20 ml) of blood, an anesthetized rabbit cardiac puncture is preferred.

5. **HAMSTER** Mesocricetus auratus is also known as the Syrian or Golden hamster. They belong to the family Cricetidae, to the subfamily Cricetinae, to the genus Mesocricetus, and the species Mesocricetus auratus. When they burrow, play, chew, and dig, the Syrian hamsters are mostly active at nighttime. Their nocturnal nature means that circadian rhythms are perfect for learning. The Syrian hamster is intensely protective, and other hamsters introduced in their environment are known to fight, bite, and even kill. Aggressive territorial behavior starts to evolve at about 8-10 weeks of age in the Syrian or Golden hamster.

Experimental Use: Among other research fields, including oncology, immunology, and physiology, the hamster is widely used in IVF research. However, their peculiar reproductive system involves a continuous 4-day estrous cycle (all non-higher primate mammals' reproductive cycle) and a very short 16-day pregnancy period.

6. **CAT** The cat (Felis catus) belongs to the Felidae (feline) family and is found on Earth in most regions except Australia and Antarctica. Usually growing to 28 inches (71 cm), they are the smallest member of the feline family. Cats are carnivores, mostly animal flesh is their diet, and they have a stomach capable of digesting raw meat. However, their diet, with the occasional hunting activities of the cat to support their diet, is typically dependent on their human owners. Though cats are not widely used in research, many diseases are similarly encountered by cats to humans. Cats also suffer from diseases such as leukemia, Alzheimer's disease, heart disease, infection, and immunodeficiency, and thus, they are excellent animal models for the mentioned diseases. Their relatively long lifespan of 20 years also makes them ideal models for illnesses and disorders that are age-related and slowly evolving. The cat has a highly developed nictitating membrane, which is contracted by stimulation of the cervical sympathetic trunk and also by several drugs like adrenaline, histamine, etc. Contraction of the nictating membrane is recorded for the investigation of ganglionic blocking agents. In cats, morphine produces excitation of the CNS.

Experimental Use: Cats are employed in acute experiments for the study of drugs affecting blood pressure. Both anesthetized and spinal preparations are used, the latter being and particularly for the

assay of catecholamines. Contractions of the nictitating membrane are recorded for the investigation of ganglionic blocking action of the drugs. Cats are essential in the study of the nerve centers in the brain because they can produce methemoglobinemia, cats are most suitable for the toxicity of compounds like acetanilide. Cats are also the model of choice for neurological research, as are studies on movement, balance, hearing, and motor neuron studies related to spinal cord injuries. They have been used for mapping trials due to anatomical similarities in brain structure. They can be also used as models for viral disease syndromes.

7. **FROG**

Frog belongs to the class of amphibians. It has been used since 200 years ago. The amphibian animal is safe to handle. It cannot breed in the lab. In India, it is the biggest frog. Rana Tigrigna's size ranges from 5 to 17 cm. They come in a variety of colors, from yellow to olive green to grey, and have dark irregular markings. Their snout is long and pointed, and their hind limbs are long and muscular. They have an average-sized head. Their toes are almost fully webbed. The eardrum is very large. The nose is slightly closer to the end of the mouth than the eye; the inter-orbital area is smaller than the upper eyelid; and the tympanum is distinct, measuring around two-thirds the size of the eye. Males have two lateral vocal sacs, which are visible externally as skin folds on the sides of the throat. Males are darker in color and have breeding pads on the first finger, but females are larger. They are most commonly used in physiology, pharmacology, and toxicology. The isolated preparation of frogs need not be maintained at 37°C and the experiments are performed at room temperature. In frogs, adrenaline is the neurotransmitter for the sympathetic nervous system.

Experimental Use: Study of a drug on the CNS, the study of isolated tissue such as rectus abdominus muscle, heart preparation, drugs acting on CNS, and drugs acting on the neuromuscular junction, as well as to determine the retinal toxicity of the drug. For several years, the African frog Xenopus laevis has been used as a biological assay to determine human pregnancy.

8. **DOG**

Dogs (Canis familiaris) belong to the Canidae family and are considered to be one of the first domesticated animals. For more than a century, they have been used in research. Dogs are carnivores but can survive in the domestic situation on a well-designed, suitably processed omnivorous diet. Dogs are useful among large laboratory animals because they can be tamed trained without much difficulty. For many reasons, dogs have been very useful research models for such a long time. One of the reasons being that dogs are physiologically quite similar to humans, they also have roughly the same number of genes as humans, and their genome has been sequenced. This makes dogs particularly useful in genetic studies. Dogs are also known to suffer from diseases such as diabetes, epilepsy, autoimmune diseases, cancers, and eye diseases that are similar to human diseases.

Experimental Uses: Acute experiment for medication affecting blood pressure and intestinal movement, gastric acid secretion research, pharmacokinetic study, antidiabetic agent study, and pharmacokinetic study. Used Tissues of Dog: Chronically prepared gastric fistula and pouches by earlier operations are also employed for the study of gastric secretion in the dog.

9. **MONKEY**

Monkeys and apes belong to the primates, the highest order of Mammals, which includes man. Most research primates are macaques or marmosets. Both structurally and functionally monkeys and apes closely resemble man. They are used in relatively limited numbers, but they've played a role in a variety of big medical breakthroughs, including the polio vaccine, premature baby life support systems, and deep brain stimulation for Parkinsonism.

Experimental Uses: Primates are used in the field of virology, parasitology, immunology, nutrition, reproduction, etc. Primate research is currently focused on infectious diseases, such as developing vaccines and therapies for HIV/AIDS. They are also used in modern drugs and vaccines for safety research.

Table: Details of Some Common Animals Used in Laboratory

Parameter	Mice	Rat	Guinea Pig	Rabbit
Scientific Name	*Mus musculus*	*Rattus norvegicus*	*Cavia porcellus*	*Oryctolagus cuniculus*
Order	Rodentia	Rodentia	Rodentia	Lagomorpha
Body Temperature	37.4°C	37.5–39°C	37.6–38.9°C	38.7–39.1°C
Respiration Rate	90–230/min	70–180/min	40–110/min	38–55/min
Heart Rate	300–750/min	260–500/min	240–400/min	135–300/min
Blood Pressure	120/75 mmHg	130/90 mmHg	75/52 mmHg	130/90 mmHg
Blood Volume (ml/kg)	55–80 (7–9%)	50–70 (≈64)	67–92 (≈75)	44–70 (≈56)
Food Consumption	15 g/100 g/day	10 g/100 g/day	6 g/100 g/day	5 g/100 g/day
Water Consumption	15 ml/100 g/day	10–12 ml/100 g/day	10 ml/100 g/day	5–10 ml/100 g/day
Life Span	1–3 years	2–3.5 years	4–5 years	4–5 (up to 15) years
Preferred Humidity	60–70%	44–60%	~45%	40–50%
Room Temperature	20–27°C	18.5–27°C	18.5–27°C	15.5–18.5°C
Mating Age	6–8 weeks	70–84 days	12–20 weeks	5–6 months

Parameter	Mice	Rat	Guinea Pig	Rabbit
Estrous Cycle	4–5 days	4–5 days	15–19 days	No regular cycle; receptivity 5–14 days
Gestation Period	19–21 days	21–23 days	59–72 days	~31 days

Table 2. Details of Some Common Animals Used in Laboratory

Parameter	Monkey	Hamster	Dog	Frog	Cat
Scientific Name	*Macaca mulatta*	*Mesocricetus auratus*	*Canis familiaris*	*Rana tigrina*	*Felis catus*
Order	Primates	Rodentia	Carnivora	Anura	Carnivora
Body Temperature	37–39 °C	36.2–37.5 °C	37.7 °C	26–28 °C	38.0–39.2 °C
Respiration Rate	76–90/min	~74/min	14–28/min	66–104/min	24–42/min
Heart Rate	Up to 150/min	280–412/min	77–138/min	~64/min	140–220/min
Blood Pressure (mmHg)	130/100	94	99/67	25–35 systolic / 18–28 diastolic	140/90
Blood Volume	54 ml/kg	78 ml/kg	86 (79–90) ml/kg	~15,93,600 cells/mm³ (female), ~10,29,700 cells/mm³ (male)	60 ml/kg
Food Consumption	1–2.4 kg/day	12 g/day	3–4 meals/day	~5 crickets per meal (0.2–0.8 g/cricket)	~40 g/kg body weight; ≥10 meals/day
Water Consumption	18.5 ml/kg/day	20 ml/day	20–70 ml/kg/day	~45 ml/kg/day	—
Life Span	~30 years	2–3 years	10–13 years	10–12 years	2–16 years
Room Temperature	37–40 °C	~37 °C	24–27 °C	18–25 °C	~21.1 °C
Mating Age	4–5 years	6–8 weeks	~90 days	~4 years	6 months (female), 8 months (male)
Estrous Cycle	26–28 days	4 days	180 days	40 days	21 days
Gestation	~165 days	15–18 days	~62 days	~33 days	58–67 days

Parameter	Monkey	Hamster	Dog	Frog	Cat
Period					
Body Weight	~5000–6000 g	110–140 g	1.5–75 kg	25–500 g	4.1–5.4 kg

ALTERNATIVES TO ANIMAL EXPERIMENTATION

In biomedical research and experimentation, alternatives or substitutes for live animals are defined as the replacement of live animals in any form, from complete to partial. To replace animal experiments, several alternative methods have been accepted around the world. The Zebrafish a recent vertebrate model, is a very effective model for measuring toxicity. Zebrafish are fastgrowing, small, and easy to keep in large numbers. Ninety percent of chemicals tested in Zebrafish triggered specific tissue, organ, and behavioral toxicity, according to reports . Chemicals may be injected directly into fish water or in small quantities by microinjections. PS1 and PS2 orthologues found in Zebrafish assist in research on Alzheimer's disease studies. It seems likely that intact animal models in pharmacology education will eventually replace these alternative methods and models, either partially or entirely. In several nations, computer-based alternatives are being used. Two versions, i.e. Expharm and Xcology, are presently available in India as free modules and advanced paying models, For years, both have been well tested and used. All countries have used computer-based alternatives to some degree. They tested these software systems and discovered that the solutions are feasible and minimize the expense and time spent on animal experiments. The students enjoyed the alternatives and considered them more effective in learning the drug action process. Although updated versions of previous software programs are available, no comprehensive revolutionary software has been released to date. International regulatory authorities have developed, validated, and/or accepted approximately 50 alternative methods and testing strategies. The three Rs are the guiding principles for using animals in scientific research in a humane manner. Any researcher who plans to use animals in their study must first show why there is no other way and what steps will be taken to reduce the number of animals used and the suffering they cause, i.e.

❖ Replacement: Methods of replacement are available. Absolute alternatives, such as in silico computer modelling [30] and in vitro methodologies are possible, as are relative substitutes that exclude or substitute the use of 'protected' animals. Examples include known animal cell lines, animal cells, tissues, and organs acquired from human-sacrificed animals, slaughter materials, invertebrates such as Drosophila and nematode worms, larval amphibians and fish, bacteria, fungi, and other microorganisms.

❖ Reduction: The use of a few animals as possible to obtain statistically meaningful outcomes and to find ways to reduce the number of animals used by each species. Only a few examples include improved experimental design and statistical analysis, new imaging methods, avoiding test repetitions to prove tested hypotheses, and data and resource sharing.

❖ Refinement Refers to reduced invasiveness, enhanced instrumentation, improved pain management that minimizes real or possible pain, discomfort, and distress. For example, non-invasive strategies involve the use of adequate pain relief anesthetic and analgesic regimes.

❖ The 4th R i.e., Rehabilitation of the animals after their use, is also emphasized.

TRANSGENIC ANIMALS

Transgenic animal is one that has been genetically modified to carry a foreign gene deliberately inserted into its genome through recombinant DNA technology. This foreign gene, along with additional regulatory sequences, is designed to ensure proper integration into the host genome and appropriate expression within the host's cells. Notable examples include transgenic sheep and goats that produce therapeutic human proteins in their milk, as well as genetically engineered chickens capable of synthesizing human proteins in the egg white. These advancements represent promising steps toward the largescale production of valuable biopharmaceuticals for use in human medicine

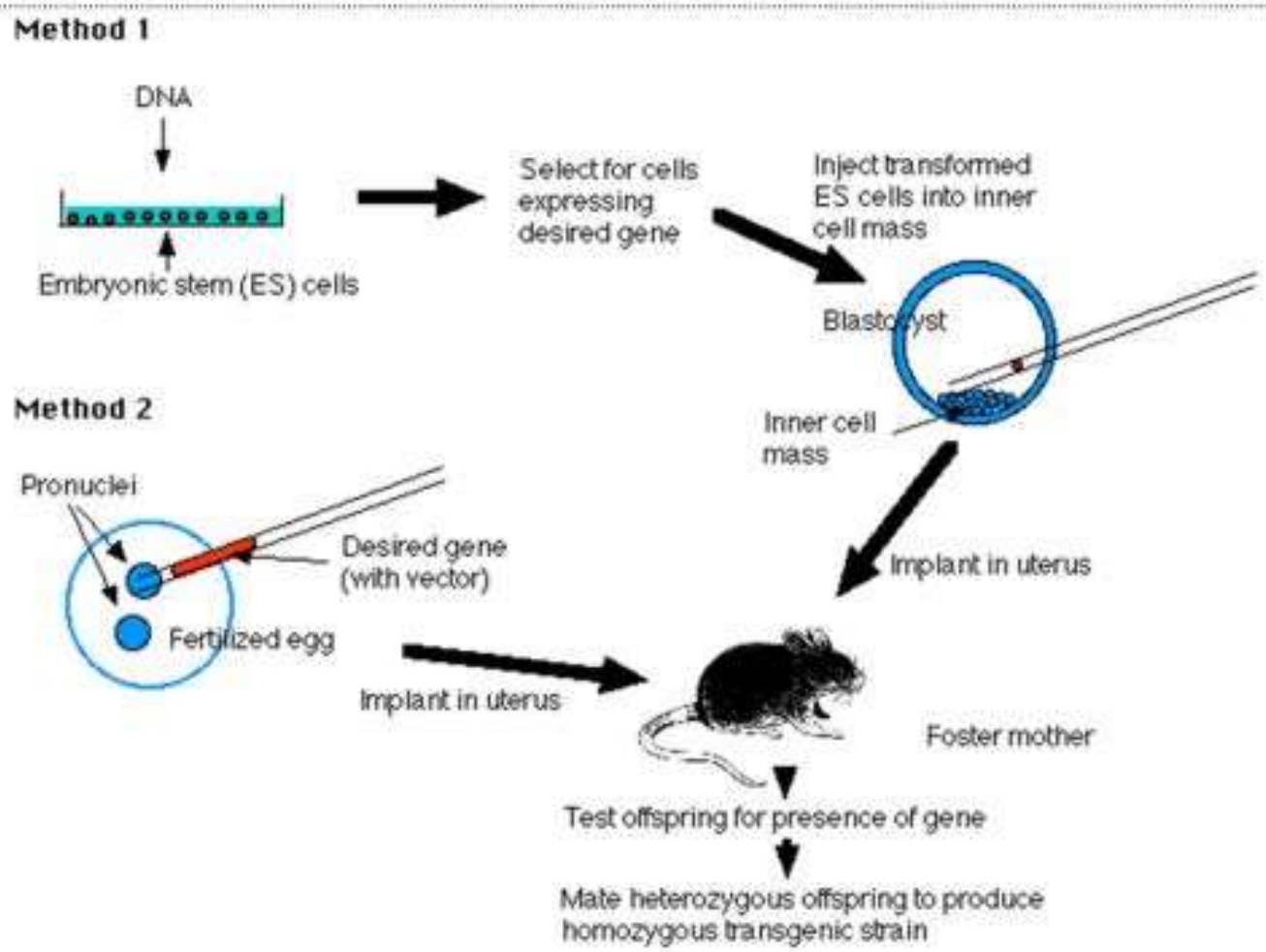
METHODS OF PRODUCING TRANSGENIC ANIMAL MODELS

Normal mice cannot be infected with polio virus. They lack the cell-surface molecule that, in humans, serves as the receptor for the virus. So normal mice cannot serve as an inexpensive, easily manipulated model for studying the disease. However, transgenic mice expressing the human gene for the polio virus receptor.

- Can be infected by polio virus and even
- Develop paralysis and other pathological changes characteristic of disease in humans.

Two methods of producing transgenic mice are widely used

- ✓ Transforming embryonic stem cells (ES cells) growing in tissue culture with desired DNA
- ✓ 2. Injecting desired gene into the pronucleus of a fertilized mouse egg



Embryonic Stem Cell Method (Method 1) Embryonic stem (ES) cells are isolated from the inner cell mass (ICM) of mouse blastocysts. These cells can be cultured in the lab and retain the potential to develop into all cell types of the mature animal, including gametes.

1. DNA Construction Using recombinant DNA techniques, scientists create a DNA molecule that contains the desired gene (e.g., the insulin gene), a vector for inserting the gene into the host's genome, and promoter/enhancer sequences to ensure proper gene expression in the host cells.

2. Transform ES Cells The engineered DNA is introduced into cultured ES cells, some of which will successfully incorporate the foreign DNA into their genome.

3. Select Transformed Cells Cells that successfully incorporate the gene are selected using specific markers.

4. Inject ES Cells into Blastocyst Transformed ES cells are injected into the ICM of a developing mouse blastocyst.

5. Embryo Transfer The modified blastocyst is transferred into the uterus of a pseudo pregnant mouse, which will foster the embryo. Only about one-third of embryos typically develop into healthy pups.

6. Test Offspring A small tissue sample from the tail of the pups is tested for the presence of the gene. Only 10–20% of pups will carry the gene and be heterozygous.

7. Establish a Transgenic Strain Heterozygous mice are mated, and their offspring are screened for homozygous individuals, which are used to establish a stable transgenic strain.

Pronucleus Method (Method 2)

1. Prepare DNA Create the desired DNA construct, as in Method 1. 2. Transform Fertilized Eggs Harvest freshly fertilized eggs before the sperm head becomes a pronucleus. Inject the male pronucleus with the prepared DNA. Once the pronuclei fuse to form a diploid zygote, allow the zygote to divide, forming a 2-cell embryo. 3. Embryo Implantation Transfer the embryos into a pseudo pregnant foster mother, following the same steps as in Method 1 for implantation and development. The offspring are then screened for the presence of the transgene.

GENE KNOCKOUT MICE

Knockout mice are genetically engineered mice in which one or more specific genes have been "knocked out" or inactivated. This is typically achieved by replacing or disrupting the target gene with a nonfunctional allele, resulting in the absence of the gene's normal function. These mice are widely used as models to study the roles of genes in health and disease, helping researchers understand gene function, the molecular basis of diseases, and potential therapeutic approaches.

How are Knockout Mice Made?

To create knockout mice, researchers use techniques such as homologous recombination in embryonic stem cells. The gene of interest is deliberately disrupted or replaced with a "null" allele, which is a version of the gene that has been rendered nonfunctional. The genetically modified stem cells are then introduced into a mouse embryo, which is implanted into a surrogate mother. The resulting offspring will carry the knockout gene in all their cells, including their germ cells, enabling the inheritance of the mutation

Generalizations from Knockout Mice Studies

1. Redundancy in the Genome: Many knockout mice show minimal effects or no noticeable changes in phenotype despite the absence of a single gene. This suggests that the mammalian genome often contains redundant or compensatory pathways that can compensate for the loss of one gene. For example, other genes or molecular pathways might take over the missing gene’s function, preventing the organism from showing a severe defect.

2. Pleiotropy of Genes: Most genes exhibit pleiotropy, meaning they have multiple functions in different tissues and at different stages of development. A single gene may have varying effects depending on the context, such as influencing growth in one organ while playing a role in immune function in another. Knockout mice provide crucial insights into the complex roles these genes play across the body.

Tissue-Specific Knockout Mice

While "housekeeping" genes are expressed in all cell types throughout development, other genes are typically expressed only in specific tissues and are activated by signals such as hormones. To study these tissue-specific genes, one might assume that traditional knockout methods would work. However, genes that are only active in certain adult tissues may still be crucial during embryonic development. In such cases, the complete knockout of the gene often leads to early embryonic lethality, preventing the study of its function. Fortunately, advances in genetic engineering have made it possible to create tissue-specific knockout mice. These mice are designed so that the gene is only knocked out in particular cell types or tissues, allowing researchers to study the gene's function in specific contexts without compromising survival.

Applications of Knockout Mice

• Gene Function Discovery - Knockout mice allow researchers to identify the roles of specific genes in development, behaviour, and disease. If a Block 3 Transgenics 10 gene is knocked out and the mouse exhibits specific physiological or behavioural changes, scientists can infer the gene's function. • Disease Modelling - Knockout mice can be used to model human diseases, especially genetic disorders caused by mutations in specific genes. By studying these mice, scientists can better understand the mechanisms behind conditions like cancer, cardiovascular diseases, neurological disorders, and metabolic diseases. • Drug Development - Knockout mice help evaluate potential therapeutic interventions by providing a way to test how a drug might impact a disease model where the target gene is absent or mutated.

Transgenic Animal	Gene Introduced / Modification	Purpose / Use	Example / Application
OncoMouse (Mouse)	Activated oncogenes related to cancer	Study of cancer development and testing anticancer drugs	Used for breast cancer and tumor research
Knockout Mouse	Specific gene removed or inactivated	Used to study gene function and disease mechanisms	Used in research of diabetes, obesity, and immune disorders
Transgenic Rat	Genes related to hypertension or neurological disorders	Used in pharmacology and toxicology studies	Model for studying hypertension and Parkinson's disease

Zebrafish	Fluorescent protein genes (GFP)	Study of embryonic development and genetic regulation	Transparent embryos help observe organ development
GloFish (Zebrafish)	Fluorescent genes from jellyfish or coral	Environmental monitoring and research	Used to detect water pollution and toxicity
Transgenic Goat	Human **antithrombin III** gene	Production of pharmaceutical proteins in milk	Drug **ATryn** used to prevent blood clots
Transgenic Sheep	Human **alpha-1 antitrypsin** gene	Production of therapeutic proteins	Treatment research for emphysema
Rosie Cow (Transgenic Cow)	Human **alpha-lactalbumin** gene	Production of human-like milk proteins	Milk enriched with human proteins for infant nutrition
Transgenic Pig	Human complement regulatory genes	Used in **xenotransplantation** research	Organs potentially used for human transplant
Enviropig	Phytase gene from bacteria	Improves digestion of phosphorus	Reduces environmental phosphorus pollution
Transgenic Rabbit	Human tissue plasminogen activator (tPA) gene	Production of blood clot dissolving proteins	Used in cardiovascular therapy research
Transgenic Chicken	Genes producing antibodies in eggs	Production of vaccines and therapeutic proteins	Antibodies harvested from egg whites
Drosophila (Fruit Fly)	Human disease genes	Study of genetics, neurobiology, and gene regulation	Research on Alzheimer's and Parkinson's disease
Transgenic Fish (Salmon)	Growth hormone gene from another fish species	Faster growth rate for aquaculture	**AquAdvantage salmon** grows faster than normal salmon

ROUTES OF DRUG ADMINISTRATION

INTRODUCTION

The choice of route for administration is governed by the nature of substances, agents, or drugs to being administered and subject that is the experimental animal to be used. The techniques vary from species to

species but to start with, research should have a general understanding of local anatomy of the site of injection. Administration of agent to experimental animals is frequently a part of experimental design, administered substances may include infectious disease agents, various therapeutics like pharmacological agents, anaesthetics, analgesics, vaccinations, antimicrobials, chemical test agents, radio contrast agents, electrolytes, other fluids and nutritive supplements. Specific considerations for delivery of substances to animals are numerous and include factors such as absorption, distribution, metabolism and excretion of therapeutic or chemical agents, route, volume, and frequency of administration, duration of treatment, pH, stability and homogeneity of the substance to be administered.

SELECTION OF A ROUTE OF ADMINISTRATION

The routes of drug administration for systemic effect may be divided into two groups enteral and parenteral, while if the drug of effect is desired locally it is administered topically, that is applied on the skin. A key factor determining the route selected is whether the agent is required to induce local or systemic effect and on the physiochemical nature of the drug.

Considering this point, a substance/drug may be given into the mouth (orally) or delivered directly into the stomach (gastric gavage), delivered into a blood vessel (intravenous), delivered into a muscle (intramuscular), into below skin (subcutaneous), instilled onto or into the eye (transcorneal or intraocular respectively), into the brain (intracerebral), administered into the peritoneal cavity (intraperitoneal), sprayed into the nose for absorption across the nasal mucous membranes or into the lungs (intranasal) or delivered into the lungs by direct tracheal instillation (intratracheal) or inhalation, or administered by a range of less common routes using other body orifices, surgical exposures, and species-specific anatomic features.

In order to get the desired effect of drug, the most important point a researcher should be well trained in the procedure for correct administration and good animal handling. Proper command leads to successful administration and varies with the routes of substance to be administered. Amongst several possibilities for the administration of substances to animal, the most common routes are intraperitoneal, intramuscular, subcutaneous, intravenous and oral administration. The intramuscular administration is not optional in case of mice if the muscle of the mouse mass is too small.

Aseptic preparation of the administration site:

The area for administration is clipped or cleaned with temperate water if necessary before cleaning the skin with disinfectant moistened cotton or alcohol. Where aseptic skin is required the fur or hair have to be clipped followed by a surgical preparation; surgical soap, alcoholic rinse and surgical preparation solution. The skin is dried immediately before administration. In some cases a local anaesthetic may be applied first to prevent pain. Test substances, solutions and equipment should be prepared aseptically and free from pyrogens, especially for parenteral injections. Living organisms or cells must be free from

contaminants when administered. The toxicity of the substance, the volume and the way of administration should be considered to prevent tissue damage and to give precise dosage.

Volume and frequency of drug administration:

The injection volume is limited by any toxicity of the substance and by the size of the animals. Administered small as possible, excess volumes of solution can shock the animal. The frequency of administration should be smallest amount, to avoid unnecessary stress. If solutions are injected iv, hemodynamic modulation and pulmonary oedema may occur while very rapid injections can produce cardiovascular failure and can be lethal (Nebendahl, 2000).

VARIOUS TYPES OF ROUTE OF DRUG ADMINISTRATION

1. **Oral Administration:**

The simplest process for administration of the drug or substance is with food or drinking water. However, this is not viable with those that are unpalatable (which cannot be swallowed) or which are not pleasant to take, insoluble or chemically unstable in drinking water or when they irritate the mucosa layer of the gastrointestinal tract (Atcha et al. 2010). In such case following techniques are commonly used to administer drugs in the experimental animals via oral route.

Intragastric administration of substances:

Direct administration or oral gavage is chosen to mixing substances with food or drinking water because the intake of substances is accurately calculated. A ball tip needle is used to prevent damaging the buccal cavity, pharynx, oesophagus and from passing through the glottal opening into the trachea. A straight line is formed between the mouth and the cardiac sphincter through the oesophageal opening. The needle is passed gently through the mouth and pharynx into the oesophagus. The mouse that usually swallows as the feeding needle approaches the pharynx, these swallowing movements can help so that the needle slide through the oesophageal opening. The substance is then administered slowlyIf any obstruction is felt, if the animal coughs, chokes or begins to struggle vigorously after the gavage begins, or if fluid is seen coming out through the nose, these may indicate that the needle has entered the lungs. Any of these signs would demand immediate withdrawal of the needle, and the mouse must be observed very carefully. If there is any sign that fluid has got into the lungs, the animal should be euthanized. As soon as administration is finished, the needle must be withdrawn (Suckow et al. 2000). This method is generally performed in rat or mice or guinea pig.

2. Parenteral Administration:

Administration of substances other than via the alimentary canal, topical application, inhalation, and implantation of an osmotic pump or a controlled release drug delivery pellet to the body includes injection, infusion. Small amounts of solution are injected, and large volumes are infused. In both cases

the skin must be penetrated by a needle. The ability to administer agents by injection is essential for most experimental studies employing laboratory animals. The commonly used routes of parenteral administration are:

(a) Subcutaneous (s.c.).

(b) Intraperitoneal (i.p.).

(c) Intravenous (i.v.).

(d) Intramuscular (i.m.).

(e) Intradermal (i.d.).

Not all techniques are appropriate for each species. For example i.m. injections are avoided in rodents especially in mouse because the amount of material that can be injected into the rodent limited muscle bunch is so small that the technique is not practical. i.p. injections are not administered to rabbits because other techniques are more appropriate.

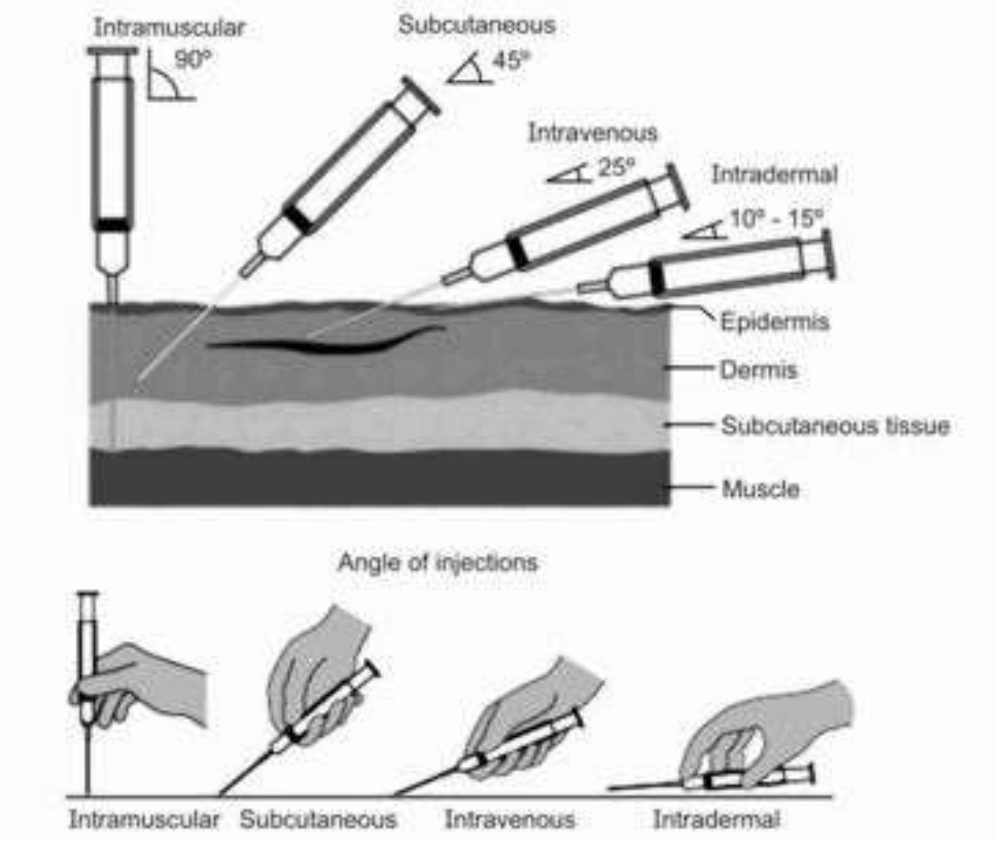


Fig: Angles of Drug Administration by different routes

3. Subcutaneous (s.c.) injection involves administering the drug into the loose skin over the interscapular or inguinal region. The technique is simple and generally not painful for a conscious animal, but the absorption rate is slower compared with intravenous or intraperitoneal injections

Technique:

1. Restrain the animal and place it on a clean surface (cotton gloves or towel).

2. Insert the needle under the skin of the inguinal area, tenting the skin with thumb and forefinger.

3. Inject the substance.

Alternatively, for mice, the animal is restrained manually with the head tilted downwards and the hind leg held firmly. The needle is inserted into the lower left or right quadrant of the abdomen, avoiding the midline. The needle should be advanced several millimeters through the subcutaneous tissue to minimize leakage .

(b) **Intraperitoneal administration:**

Injection into the abdominal cavity or peritoneal cavity is a common technique in experimental animals but infrequently used in larger mammals. Intraperitoneal injection is used for small species for which intravenous administration is challenging and it can be used to administer large volumes of fluid safely (10 ml/kg). This is the most common route being as it is technically simple and easy. Boundaries are the sensitivity of the tissue to irritating substances, less tolerance to solutions of non-physiological pH.

Technique:

The animals are manually controlled and are held in a supine position with its posterior end slightly elevated or the head can be tilted lower than the body The needle and syringe should be kept almost parallel to its vertebral column in order to avoid accidental penetration of the urinary bladder or intestines or gut The needle is pushed in at an approximately 10° angle between the needle and the abdominal surface in the lower right abdominal quadrant To avoid leakage from the puncture point, the needle is run through subcutaneous tissue in a cranial direction for 2-3 mm and then inserted through the abdominal wall. The recommended volume is maximum of 10 ml/kg For intraperitoneal injections in fish, the animals are controlled on their side on a flat surface, and the needle should enter along the midline just anterior to the pelvic fins. Larger fish may require sedation or light anesthesia for appropriate self-discipline

Precautions:

Drugs or agents or chemicals or solution administered intraperitoneally should be sterile, isotonic, and non-irritating. Irritating substances injected intraperitoneally may induce painful ileus and peritonitis

(inflammation in peritoneal cavity) in experimental animals, with subsequent adhesions. Although technically a simple procedure to perform, training and competency of personnel should be monitored to ensure that substances are delivered accurately.

(c) **Intravenous administration:**

Intravenous injections are usually made into the lateral tail veins (not the dorsal tail vein) because the dorsal vein is not straight. The lateral veins are readily visualized but have quite small diameters. If anesthesia is not used, a restraining device is necessary for handling and restraint.

Technique:

To dilate the vein, the mouse is either placed in a restrainer or anesthetized and the tail is then warmed with a lamp or cotton, or immersed in warm water (40-45 °C). The tail is swabbed with 70 % alcohol on a gauze sponge or swab. Insert the needle parallel to the tail vein, penetrating 2-4 mm into the lumen while keeping the bevel of the needle face upwards. The solution is then injected slowly; no resistance should be felt if the solution is properly administered. When the intravenous administration is finished or the cannula is pulled out, the injection site must be pressed firmly with a swab or fingers to prevent backflow of the administered solution or blood. The site of administration for i.v. injection in mice is shown in Fig. 4.5.

Other sites for i.v. administration:

The ophthalmic plexus route is also used for intravenous administration. The method resembles blood collection by retro-orbital sinus puncture. The mouse is anesthetized and then manually restrained on a solid surface, being held softly but tightly by the nape of the neck. By pressing down with the thumb and forefinger in the occipital area and pulling back the skin, the point of the needle can be directed toward the back of the orbit at a slight angle20–40° angle. The needle is inserted medially through the conjunctiva on the inner side of the ocular cavity. If entry is blocked by bone, the needle is withdrawn slightly. Fluid is injected slowly loosening the skin slightly. Other routes for intravenous administration via the external jugular vein, the dorsal metatarsal vein and the sublingual vein have been reported

Precaution:

If the same vein is needed to be used several times, the first administration should be made as distal as possible in relation to the heart and subsequent administrations should be placed progressively more proximally. Because vein puncture and the administration of substances can damage or block the vein, the distal part of vein may no longer be used .

(d) **Intramuscular administration:**

Intramuscular injections may be painful because muscle fibres are necessarily placed under tension by the injected material. Sites need to be chosen to minimize the possibility of nerve damage. Sites should be rotated for multiple dose studies. A distinction needs to be made between aqueous and oily formulations when speed of absorption is important (oily formulations are likely to remain as a depot for 24 h). With multiple dose studies there is a need to consider the occurrence of inflammation. This should usually be avoided, in mice because mouse muscles are small. If necessary, it may be given into the thigh muscle with injection volumes less than 0.05 ml.

Technique:

The tip of needle should be directed away from the femur and sciatic nerve. The rat is anesthetized or is manually restrained by another person. The needle tip is inserted through the skin and into the muscle. If back flow of body fluid or blood, stop the procedure. The needle must be moved or a fresh attempt must be made. Good technique and restraint are necessary for intramuscular administration and should only be performed by well trained persons

(e) **Intradermal administration:**

This site of administration is commonly should be restricted to cases of absolute necessity. It is very difficult in the rodent (mice and rats) due to the very thin skin. Using a fine needle with 29 G or lesser in size is suggested.

Technique:

The animal is anesthetized, the hair or fur clipped or removed from an area on the back, hind footpad, ventral abdomen, which is wiped with 70–75% ethanol on a cotton. The skin is held tautly with thumb and index finger and the needle inserted, bevel up and at a shallow angle, just under the superficial layer of epidermis. The volume should be 0.05–0.1 ml can be used, dependent upon the thickness of the skin per site. Resistance should be felt both as the needle is advanced and as the compound is injected. A hard bleb will be seen upon successful intradermal injection of even a small quantity of fluid (Suckow et al., 2000). If multiple sites are injected, adequate separation is necessary to prevent coalescing of lesions.

Intranasal Administration:

These are generally performed with the animals lightly anesthetized.

Technique:

The animals are manually controlled and the tail anchored between the small finger and the palm. The rat is held in a supine place with the head elevated. The end of the micropipette is placed at or in the

external nostrils, and then the drugs or solution is poured in slowly. The volume should be less than 0.02 ml; excess volume or rapid injection will induce suffocation and death.

4. Topical Administration or Application of Sustenance:

The back and abdominal region of the animal is normally the site of topical administration of drugs, topical administration not a frequently realized that the cutaneous or skin is the largest organ of the body and survival depends on its patency perhaps more than for most other organs. The skin is also a convenient site for the administration of drugs. Numerous factors, such as the physicochemical properties of the drugs, the attributes of the vehicle and the permeability of the skin, can affect the degree of percutaneous absorption (Franklin et al., 1989). The capability of an agent to be absorbed via skin and enter the systemic circulation is determined by its ability to partition into both lipid and water phases.

Technique:

After clipping the hair, the hairless region should be cleaned from any oily and debris. The drugs or substance should be dissolved in a suitable volatile solvent or mixed in a suitable cream before application and then applied with a dropper or smeared onto the skin with a swab. Precautions are required to be taken to prevent the animal from licking or scratching the application sites.

SPECIALIZED TECHNIQUES OF DRUG ADMINISTRAON

Pharmacological parenteral methods focusing on implantable osmotic pumps, controlled-release drug delivery pellets, and implantable cannulas.

1. Osmotic pump:

- Used for systemic administration when implanted subcutaneously, intraperitoneally, or via catheter for intravenous, intracerebral, or intra-arterial infusion.

- Enables targeted delivery to sites like spinal cord, spleen, liver, organ transplants, or wound-healing areas.

- Works by releasing substances at a slow, steady rate over days/weeks/months without external connections.

2. Controlled-release drug delivery pellets:

- Pellets continuously discharge the active product in the treated animal.

- Intended for subcutaneous implantation (or other routes) in laboratory animals.

3. Implantable cannula:

- Provides continuous venous or arterial access for substance administration or blood withdrawal.

- Inserted into a vein/artery (carotid artery, femoral vessels, jugular vein) with a subcutaneous port usually at the shoulders region.:

- (A) Osmotic pump working method: components include a salt chamber, water chamber, hollow fiber, movable barrier, drug reservoir, and drug outlet with a semi-permeable wall.

- (B) Osmotic pump design: shows an osmotic actuator, movable plunger, water cartridge, and standard syringe packaging with dimensions (35 mm × 85 mm, 3.0 mL capacity).

(d) **Intracerebroventricular (I.c.v.) Injection:**

The study involving the I.c.v. administration in animal (rat and mouse), a cannula was implanted into the right lateral ventricle of animal. Animals were anesthetized and fixed in a stereotaxic frame. A 24-gauge stainless steel guide cannula was implanted aseptically into the right lateral ventricle (coordinates from Paxinos and Franklin 2001, posterior –0.22 mm; lateral from midline +1.2 mm and ventral –2.0 mm relative to bregma). The guide cannula was secured in place by dental cement (Dental Products of India, Mumbai) affixed to two mounting screws. The stainless steel dummy cannula was used to occlude the guide cannula when not in use. Moreover, during the phase of recovery from i.c.v. surgery to 5-7 the cannulated animals were handled daily during the recovery period and habituated for aCSF injections to minimize the effect of isolation or non-specific stress. Injections were made using a Hamilton microliter syringe connected to internal cannula (30 gauges) by polyethylene tubing and a final volume of 5 µl was administered over a period of 1 min into the right lateral ventricle. The injection cannula was left in place for further 1 min before being slowly withdrawn to avoid back flow

BLOOD COLLECTION

Collection of blood from small laboratory animals is necessary for a wide range of scientific research and there are a number of efficient methods available for that. It is important that blood sample collection from experimental animals should be least stressful because stress will affect the outcome of the study. Various regulatory agencies and guidelines have restricted the use of animals and the techniques used for blood collection in laboratory animals. This article deals with the approved blood collection techniques for laboratory animals like rodents, lagomorphs and non rodents. Permission of the Institute Animal Ethics Committee has been obtained for the use of animals for demonstrating the techniques.

GENERAL PRINCIPLES OF BLOOD COLLECTION IN ANIMALS

- The method of blood collection should be described in the protocol approved by the Institute animal ethics committee.
- It should be least painful and stressful. Blood sample may be collected under anesthesia or without anesthesia.
- Adequate training is required for blood collection using any method in any species.

In general, blood sample is withdrawn from venous, arterial blood vessels or heart chambers. Frequency of blood collection is important. Once in two weeks is ideal for nonrodents. If the study needs multiple blood samples, lagomorphs (e.g., hares and rabbit) can be used. All nonterminal blood collection without replacement of fluids is limited up to 10% of total circulating blood volume in healthy, normal, adult animals on a single occasion and collection may be repeated after 3 to 4 weeks. In case repeated blood samples are required at short intervals, a maximum of 0.6 ml/kg/day or 1.0% of an animal's total blood volume can be removed every 24 hour.

If the study involves repeated blood sample collection, the samples can be withdrawn through a temporary cannula. This may reduce pain and stress in the experimental animals. • The estimated blood volume in adult animals is 55 to 70 ml/kg body weight. Care should be taken for older and obese animals. If blood collection volume exceeds more than 10% of total blood volume, fluid replacement may be require

Lactated Ringer's solution (LRS) is recommended as the best fluid replacement by National Institutes of Health (NIH). If the volume of blood collection exceeds more than 30% of the total circulatory blood volume, adequate care should be taken so that the animal does not suffer from hypovolemia

Table 1: Commonly recommended anesthetic agents for laboratory animal experiments

Animal species	Short anesthesia	Medium anesthesia	Long anesthesia
Mice	Isoflurane (inhalation)	Xylazine + ketamine	Xylazine + ketamine
	Halothane (inhalation)	(5 mg + 100 mg i.m.)	(16 mg+60 mg i.m./i.p.)
Rat		Xylazine + ketamine	Xylazine + ketamine
		(5 mg + 100 mg i.m.)	(16 mg +60 mg i.m./ i.p.) or
			Urethane (1200 mg/kg i.p.)
Guinea pig	Isoflurane (inhalation)	Xylazine + ketamine	Xylazine + ketamine
		(2 mg + 80 mg i.m.)	(4 mg + 100 mg i.m.)
Rabbits	Isoflurane (inhalation)	Xylazine + ketamine	Xylazine + ketamine
		(5 mg + 15 – 30 mg i.m.)	(5 mg + 100 mg i.m.)

GENERAL METHODS FOR BLOOD COLLECTION

Blood samples are collected using the following techniques:

- Blood collection not requiring anesthesia *ƒ* Saphenous vein (rat, mice, guinea pig) *ƒ* Dorsal pedal vein (rat, mice)

- Blood collection requiring anesthesia (local/general anesthesia) *ƒ* Tail vein (rat, mice) *ƒ* Tail snip (mice) *ƒ* Orbital sinus (rat, mice) *ƒ* Jugular vein (rat, mice) *ƒ* Temporary cannula (rat, mice) *ƒ* Blood vessel cannulation (rat, guinea pig, ferret) *ƒ* Tarsal vein (guinea pig) *ƒ* Marginal ear vein/artery (rabbit)
- Terminal procedure *ƒ* Cardiac puncture (rat, mice, guinea pig, rabbit, ferret) *ƒ* Orbital sinus (rat, mice) *ƒ* Posterior vena cava (rat, mice)

PROCEDURE FOR SAPHENOUS VEIN BLOOD SAMPLE COLLECTION

- Requirements include animal, rodent handling gloves, towel, cotton, sample collection tubes and 20G needle.
- Lateral saphenous vein is used for sampling while taking aseptic precautions.
- The back of the hind leg is shaved with electric trimmer until saphenous vein is visible. Hair removal cream can also be used.
- The animal is restrained manually or using a suitable animal restrainer.
- Hind leg is immobilized and slight pressure may be applied gently above the knee joint.
- The vein is punctured using a 20G needle and enough volume of blood is collected with a capillary tube or a syringe with a needle.
- The punctured site is compressed to stop the bleeding.
- While collecting blood: *ƒ* the local anesthetic cream may be applied on the collection site no more than three attempts are made, continuous sampling should be avoided and collecting more than four samples in a day (24-hour period) is not advisable.

PROCEDURE FOR DORSAL PEDAL VEIN BLOOD SAMPLE COLLECTION

Requirements include animal (rat or mice), rodent handling gloves, cotton, capillary tube, 23G/27G needle and blood sample collection tubes.

• The animal is kept in a restrainer.

• The hind foot around ankle is held and medial dorsal pedal vessel is located on top of the foot.

• The foot is cleaned with absolute alcohol and dorsal pedal vein is punctured with 23G/27G needle.
• Drops of blood that would appear on the skin surface are collected in a capillary tube and a little pressure is applied to stop the bleeding

PROCEDURE FOR TAIL VEIN BLOOD SAMPLE COLLECTION

Requirements include animal, rodent handling gloves, towel, cotton, sample collection tube and animal warming chamber.

• This method is recommended for collecting a large volume of blood sample (up to 2ml /withdrawal)

• The animal is made comfortable in a restrainer while maintaining the temperature around at 24 to 27°C.

• The tail should not be rubbed from the base to the tip as it will result in leukocytosis. If the vein is not visible, the tail is dipped into warm water (40°C).

• Local aesthetic cream must be applied on the surface of the tail 30 min before the experiment.

• A 23G needle is inserted into the blood vessel and blood is collected using a capillary tube or a syringe with a needle. In case of difficulties, 0.5 to 1 cm of surface of the skin is cut open and the vein is pricked with bleeding lancet or needle and blood is collected with a capillary tube or a syringe with a needle.

• Having completed blood collection, pressure/silver nitrate ointment/solution is applied to stop the bleeding.

• If multiple samples are needed, temporary surgical cannula may be used.

• Restrainer is washed frequently to avoid/prevent pheromonally induced stress or cross infection

PROCEDURE FOR TAIL SNIP BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, surgical blade and blood sample collection tubes.

• This method is recommended for blood collection only in mice.

• This method should be avoided as far as possible because it can cause potential permanent damage on the animal tail. If needed, it should be done under terminal anesthesia only.

• Before collecting the blood, local anesthesia is applied on the tail and a cut is made 1 mm from the tip of the tail using scalpel blade. • Blood flow is stopped by dabbing the tail tip.

ROCEDURE FOR ORBITAL SINUS BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, capillary tube and blood sample collection tubes.

• This technique is used with recovery in experimental circumstances and this method is also called periorbital, posterior-orbital and orbital venous plexus bleeding. • Blood sample is collected under general anesthesia. • Topical ophthalmic anesthetic agent is applied to the eye before bleeding. • The animal is scruffed with thumb and forefinger of the nondominant hand and the skin around the eye is pulled taut. A capillary is inserted into the medial canthus of the eye (30 degree angle to the nose). • Slight thumb pressure is enough to puncture the tissue and enter the plexus/sinus. • Once the plexus/sinus is punctured, blood will come through the capillary tube. • Once the required volume of blood is collected from plexus, the capillary tube is gently removed and wipedwith sterile cotton. Bleeding can be stopped by applying gentle finger pressure. • Thirty minutes after blood collection, animal is checked for postoperative and periorbital lesions •

Caution:

- Repeated blood sampling is not recommended.
- Skill is required to collect blood.
- Even a minor mistake will cause damage to the eyes.
- Two weeks should be allowed between two bleedings.
- Adverse effects reported from this method is around 1 to 2% which includes hematoma, corneal ulceration, keratitis, pannus formation, rupture of the globe, damage of the optic nerve and other intraorbital structures and necrotic dacryoadenitis of the harderian gland.

PROCEDURE FOR JUGULAR VEIN BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, 25G needle and blood sample collection tubes.

In this method, warming of the animals is not required and is used to collect micro volumes to one ml of blood sample. This method has to be carried out under general/inhalation anesthesia and two persons are needed to collect blood sample. • One person has to restrain the animal and monitor the animal. Another person is required to collect the blood sample from the animal. The neck region of the animal is shaved and kept in hyperextended position. The jugular veins appear blue in color and is found 2 to 4 mm lateral to sternoclavicular junction. A 25G needle is inserted in the caudocephalic direction (back to front) and blood is withdrawn slowly to avoid collapse of these small blood vessels. Animal has to be handled carefully and not more than 3 to 4 mm of needle is to be inserted into the blood vessel. If the attempt to collect blood fails, the needle is slowly removed and the site is monitored for bleeding. If there is no bleeding, one more attempt can be made. Further attempts should be avoided in case of bleeding as it may collapse the vein. Finger pressure is applied to stop bleeding.

Caution: *f* Number of attempts is limited to three. *f* Apply local anesthetic cream 30 minutes prior to sampling.

PROCEDURE FOR BLOOD SAMPLE COLLECTION WITH TEMPORARY CANNULA

Requirements include animal, anesthetic agent, cotton, 25G needle, animal warming chamber and blood sample collection tubes. Usually a temporary cannulation is made in the tail vein and used for a few hours. The animal is restrained and local anesthetic cream is applied on the tail (1 – 2 cm above the tail tip). The tail is either cannulated or a 25G needle is used. Tail bleeding normally requires the animal to be warmed in order to dilate the blood vessels (37 – 39°C for 5 – 15 min). After cannulation, animal has to be housed individually in large cages.

PROTOCOL FOR BLOOD VESSEL CANNULATION

Requirements include animal, anesthetic agent, cotton, 25G needle, i.v. cannula, surgical blade, heparin (or any anticoagulant) and blood sample collection tubes. This method involves continuous and multiple sampling in the experimental animal. • This method requires close and continuous monitoring of the animal. Usually blood vessel cannulation is done in the femoral artery, femoral vein, carotid artery, jugular vein, vena cava and dorsal aorta. Surgery is required for this method and appropriate anesthesia and analgesia should be used to minimize the pain. After surgical cannulation, animal should be housed singly in a large and spacious cage. Blood sample may be collected over 24 hour at the volume of 0.1 to 0.2 ml/sample. After withdrawing the blood, the cannula is flushed with an anticoagulant and the withdrawn volume may be replaced (if required) with LRS and cannula should be closed tightly

Caution: The experiment has to be conducted fully under aseptic precautions. Infection, hemorrhage, blockage of cannula and swelling around the cannulation site should be looked for.

Table 2: Needle size used for blood vessel cannulation in different species

Species	Needle to be used	Maximum collection volume
Mice	23 – 25G	1 ml
Rat	19 – 21G	10 – 15 ml
Rabbit	19 – 21G	60 – 200 ml
Guinea pig	20 – 21G	1 – 25 ml

PROTOCOL FOR TARSAL VEIN BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, 22G needle, hair remover and blood sample collection tubes. Tarsal vein is identified in one of the hind legs of large animals. This method is commonly recommended for guinea pig. One person has to restrain the animal properly. Tarsal vein may be visible in blue color. The surface hairs are removed by applying a suitable hair remover. A local anesthetic cream is applied on thecollection site. After 20 to 30 minutes, blood sample is collected slowly by using 22G needle. Maximum three samples can be taken per leg and 0.1 to 0.3 ml of blood can be collected per sample. After the sample collection, gentle pressure is applied with finger for 2 minutes to stop bleeding

Caution: Not more than six samples from both hind legs are taken. *f* The number of attempts is three or less.

PROTOCOL FOR MARGINAL EAR VEIN/ ARTERY BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, 26G needle, 95% v/v alcohol, o-Xylene, surgical blade and blood sample collection tube.

• This method is commonly adopted for rabbits.

• The animal should be placed in a restrainer. Ear is cleaned with 95% v/v alcohol and local anesthetic cream is applied on the collection site 10 min prior to sampling. (If required, the o-Xylene/topical vasodilator may be applied topically on the collection site to dilate blood vessels). Size 11 surgical blade is used to cut the marginal ear vein and blood is collected in a collecting tube. Otherwise, a 26G needle may be used to collect blood from animal marginal vein. After collecting blood, clean sterile cotton is kept on the collection site and finger pressure is applied to stop the bleeding

PROTOCOL FOR CARDIAC PUNCTURE

Requirements include animal, anesthetic agent, towel, cotton, 19 to 25G needle with 1 to 5 ml syringe, surgical blade, tube (internal diameter of 0.1 to 0.3 mm) for thoracotomy, plastic disposable bag and blood sample collection tubes.

In general, cardiac puncture is recommended for terminal stage of the study to collect a single, good quality and large volume of blood from the experimental animals.

• During blood sample collection, animal will be in terminal anesthesia.

• Appropriate needle is used for blood sample collection with or without thoracotomy. Blood sample will be taken from the heart, preferably from the ventricle slowly to avoid collapsing of heart

• Caution: If animal has dextrocardia, sampling may fail

PROTOCOL FOR BLOOD SAMPLE COLLECTION THROUGH POSTERIOR VENA CAVA

Requirements include animal, anesthetic agent, surgical blade, small glass rods, surgical scissor, 21 to 25G needle with 1 to 5 ml syringe and blood sample collection tube.

In general, posterior vena cava blood sample is recommended for terminal stage of the study. Animal have to be anesthetized and 'Y'- or 'V'-shaped cut in the abdomen is made and the intestines are gently removed. The liver is pushed forward and the posterior vena cava (between the kidneys) is identified. 21 to 25G needle is inserted to collect blood from the posterior vena cava. • This procedure will be repeated three to four times to collect more volume of blood sample.

ANESTHESIA AND EUTHANASIA

A fundamental responsibility of individuals that use animals in research, teaching or testing is to anticipate and eliminate or minimize any potential that procedures may cause animal pain, distress, or discomfort. • Although animals that are in pain may not behave like humans, (e.g., pain in animals may be accompanied by immobility and silence, in contrast to the groans and cries of human patients), it is assumed that procedures that cause pain in humans cause pain in animals.

The presence of pain in animals can be recognized by alterations in animal behavior (e.g., reduced activity, reduced grooming, hunched- up posture, altered gait, changes in temperament, vocalizations, reduced food and water intake, reduced urinary and fecal output), and in physiological variables, (e.g., reduced depth of respiration, increased heart rate, and reduced hydration status).

Animal pain, distress, and discomfort can produce a range of undesirable physiological changes, which may radically alter measured responses to experimental stimuli, as well as the rate of recovery from surgical procedures, hence, its avoidance and alleviation are in the best interest of both the animal and researcher. • Reducing post-procedural/post-operative pain, distress, and discomfort is accomplished by good nursing care, (e.g., keeping the animal warm, clean, dry and well padded), and by the administration of analgesic drugs. • In addition to the avoidance and alleviation of pain and discomfort, adequate post-procedural /postoperative animal care also includes efforts to prevent and/or treat post-anesthetic complications, (e.g., aspiration, hypostatic pneumonia, cardiovascular and respiratory depression, dehydration, and infection).

The prevention or minimization of animal pain, distress, or discomfort by the proper use of tranquilizers, anesthetics, and analgesics is scientifically and ethically essential to the humane care, use, and treatment of research animals. The use of these classes of drugs must effectively prevent or minimize suffering and discomfort of animals during potentially painful procedures.

The use of these three classes of drugs must be in accordance with currently accepted veterinary medical practice and produce in the subject animal an appropriate level of tranquilization, anesthesia, or analgesia consistent with the protocol or design of the experiment.

DEFINITIONS:-

• Neuroleptic - produces central nervous depression, depression of excitability of the autonomic nervous system, a dulling of consciousness and a reduction of spontaneous motor activity (e.g., tranquilizers/sedatives).

• Analgesia - relief from pain. • Preemptive analgesia - managing pain before it begins.

• Tranquilization - a state of behavioral change in which the patient is relaxed, unconcerned by its surroundings, and often, indifferent to minor pain.

• Sedation - a mild degree of central depression in which the patient is awake but calm; larger doses of sedative may lead to narcosis.

• Narcosis - a drug-induced state of sedation in which the patient is oblivious to pain. • Local anesthesia - loss of sensation in a limited body area.

• Regional anesthesia – loss of sensation in a larger, though limited, body area. • Basal anesthesia - a light level of general anesthesia usually produced by preanesthetic agents; serves as a basis for deeper anesthesia following the administration of other agents.

• General anesthesia - complete unconsciousness • Surgical anesthesia - unconsciousness, accompanied by muscular relaxation to such a degree that surgery can be performed painlessly.

• Neuroleptanalgesia - a state of central nervous system depression and analgesia usually produced by a combination of a neuroleptic and a narcotic analgesic.

GENERAL CONSIDERATIONS

In order to reduce anesthetic risk and prevent post-anesthetic complications, animals must first be examined for signs of disease or distress including, but not limited to, ruffled, matted or dull hair coat, labored breathing, lack of inquisitiveness, failure to respond to stimuli, abnormal posture/positioning, dehydration, or impaired locomotion. Acclimatizing animals allows them to adjust physiologically and psychologically to their new environment and provides the opportunity to carefully monitor for any abnormalities. Animals should be acclimatized for a minimum of 7 days.

When planning to administer drugs, recall that dosage charts for anesthetic and analgesic agents state only the average amount of drug that would be expected to produce a desired level of anesthesia or

analgesia under standard conditions. Consequently, animals must be monitored carefully and the dosages tailored to meet each clinical and research situation. The duration of anesthesia produced by the anesthetic should coincide with the expected duration of the operative procedure.

ANAESTHESIA

The word anaesthesia has been derived from Greek word that means ―without perception of insensibility‖.

Anesthesia is the act of providing sensation-free relief from pain or pain-producing procedures. • Anesthesia must be performed by a person with knowledge of and familiarity with the drugs to be used in the animal species under consideration.

FACTORS AFFECT THE ACTIVITY OF ANAESTHETICS

Many factors can affect the activity of anesthetics. The species, strain, sex, age, nutritional and disease status, relative body size, disposition/demeanor, presence of concurrent pain or distress, or medication are known to cause a variation in the amount of drug needed to produce a desired effect in an individual animal. Commonly used laboratory anaesthetics

There are numerous anesthetics available for use in rodents. Some of the more popular agents include: ⎪Chloralose ⎪Urethane ⎪Barbiturates ⎪Paraldehyde ⎪Magnesium Sulphate ⎪Ketamine ⎪Tribromoethanol

CHLORALOSE

It is a compound of chloral and glucose prepared by heating equal parts of anhydrous glucose and charcoal, when both α- chloralose (active form) and β-chloralose (in active form) are formed. • It is prepared as one percent solution by boiling in 0.9% NaCl or in distilled water, and administered intravenously or intraperitoneally at a temperature of 30-400C before the chloralose comes out of solution.

Disavantages:- It is suitable only for acute experiments, usually in dogs and cats, inducing surgical anaesthesia for 3-4 hours or longer. Advantages:- It has the advantage of greater constancy of the depth of anaesthesia. The respiration and circulation are not depressed, and the blood pressure is well maintained usually on the higher side. Reflexes are not depressed but may be slightly exaggerated including responses to bilateral carotid occlusion.

Dog- 1% aq. Solution (hot) 80-120mg i.v. 10% in polyethylene glycol 100mg i.v.

Cat- 1% aq. Solution (hot) 80mg i.v. 2% aq. Or saline suspension 80-100mg i.p. 10% in propylene glycol 100mg i.p.

Rat- 10% in propylene glycol 80mg i.p.

URETHANE (Ethyl Carbamate)

It is readily soluble in water giving a neutral solution. Usually 25% solution in water is used. Disadvantages:- • It is suitable only for acute experiments since it has delayed toxic effect on liver, and may also cause agranulocytosis and pulmonary adenomata.

Mice develop an exceptionally high incidence of lung tumours regardless of the route of administration. Dog- 25% aq. Solution 1.5g i.v.

Cat- 25% aq. Solution 1.0-1.5 g i.v.

Rabbit- 25% aq. Solution 0.5 – 1.75g i.v.

50% aq. Solution 1.5-2g i.p

Guinea pig- 25% or 50% aq. Solution 1.5g i.p.

Rat- 25% aq. Solution 1.25-1.75g i.m. or s.c. 20% aq. Solution 1.5g i.p.

BARBITURATES

Barbiturates interfere with nerve impulse transmission both in the central nervous system and in the ganglia producing depression of cardiovascular and spinal cord reflexes. • In rabbits pedal reflex (leg retraction) is lost first, then pupillary and finally palpebral reflex.

Pentobarbital

Pentobarbital is a barbiturate and, historically, the most commonly used anesthetic in rodents.

Advantages:- At recommended doses, it causes minimal cardiovascular depression. It is also relatively long acting and can provide approximately 45 minutes of surgical anesthesia.

Disadvantages:- Pentobarbital is a potent inducer of the hepatic microsomal enzyme system. Causes pronounced respiratory depression as well as hypothermia, particularly when repeated doses are given. Pentobarbitone sodium

Monkey- 6% solution 25mg i.p. or i.v.

Dog and cat 6% solution 30-50mg i.v. or i.p.

Rabbit – 6% solution 50-60mg i.v.

Guinea pig 1% solution 30-50mg i.p.

Rat and mouse 0.6% solution 30-60mg i.p.

Frog – 0.6% solution 50mg intraabdominally.

Phenobarbitone sodium: Phenobarbitone sodium and barbitone sodium are used for prolonged experiments.

Dog and cat – 10% aq. Solution 180-200mg i.p.

Tiopentone sodium: Thiopentone sodium (pentothal) is used for surgical operations of short duration. It produces rapid induction with minimum excitation.

Dog – 2.5% fresh solution. 12-16 mg i.v. (for brief duration) 20-26mg i.v. (for longer duration)

PARALDEHYDE

Advantages:- It has a wide margin of safety because it depresses only the cerebrum and not the medullary centres. Intravenous injection is likely to produce cardiac dilatation and pulmonary congestion and oedema.

Disadvantages:- Under its influence the basal blood pressure as well as the response to vasopressor and depressor drugs are low. Bilateral carotid occlusion produces poor pressor response or even a depressor response.

Dog – 6% solution 1.2ml i.p

Cat – 6% solution 2.1ml i.m.

MAGNESIUM SULPHATE

A 20% magnesium sulphate solution 5ml/kg intravenously produces anaesthesia for about an hour. Calcium gluconate intravenously will counteract its depressant effect immediately. Its principal use is in producing euthanasia.

Tribromoethanol

Advantages:- In most rodents, tribromoethanol produces good surgical anesthesia, with good skeletal muscle relaxation and only a moderate degree of respiratory depression. It is relatively inexpensive and not a controlled agent.

Disadvantages:- It is a potential for causing peritonitis. When exposed to either light or temperatures >400C, tribromoethanol degrades into two byproducts: hydrobromic acid and dibromoacetaldehyde. Both of these compounds are highly irritating when administered IP and result in peritonitis and visceral adhesions which may be fatal.

Ketamine hydrochloride

Ketamine hydrochloride, a dissociative anesthetic, disrupts pain transmission and suppresses spinal cord activity with some action at opioid receptors. Visceral pain is not abolished with dissociative anesthetics and there is poor muscle relaxation and analgesia.

Disadvantages:

Ketamine is a poor anesthetic when used alone, but is more often combined with other agents. When combined with other drugs, it is usually administered IP. Ketamine is acidic, can be irritating, and cause muscle necrosis when administrated IM. Ketamine-induced nerve damage can cause selfmutilation in rodents. Ketamine is a controlled substance. Store in a locked cabinet and maintain a log of its use.

EUTHANASIA

The term euthanasia is derived from the Greek terms meaning good and thanatos meaning death.

A good death‖ eu would be one that occurs with minimal pain and distress. In the context • Euthanasia is the act of inducing humane death in an animal. —Sacrificing the experimental animal after use by gentle procedure causing minimum of physical and mental suffering is called euthanasia (Painless killing). Objectives of euthanasia:-

The primary criteria for euthanasia in terms of animal welfare are that the method be painless, achieve rapid unconsciousness and death, require minimum restraint, avoid excitement, is appropriate for the age, species, and health of the animal, must minimize fear and psychological stress in the animal, be reliable, reproducible, irreversible, simple to administer (in small doses if possible) and safe for the operator, and, so far as possible, be aesthetically acceptable for the operator.

Methods of Euthanasia

Methods of euthanasia fall into two broad categories.

- *Chemical methods*- Inhalant agents:- Ex – ether, halothane, methoxyflurane, isoflurane, enflurane, chloroform, nitrogen, nitrous oxide, carbon di oxide, carbon monoxide, argon, hydrogen cyanide. • Injectable agents:- Ex – barbiturates, chloral hydrate, ethanol, ketamine, magnesium sulphate, potassium chloride, neuromuscular blocking agents.
- *Physical methods*. : PENETRATING CAPTIVE BOLT • EUTHANASIA BY A BLOW TO THE HEAD • GUNSHOT • CERVICAL DISLOCATION • DECAPITATION • ELECTROCUTION • MICROWAVE IRRADIATION • THORACIC (CARDIOPULMONARY, CARDIAC) COMPRESSION • KILL TRAPS • MACERATION • ADJUNCTIVE METHODS • Exsanguination • Stunning • Pithing

PENETRATING CAPTIVE BOLT

A penetrating captive bolt is used for euthanasia of ruminants, horses, swine, laboratory rabbits, and dogs. Its mode of action is concussion and trauma to the cerebral hemisphere and brainstem.

Advantages— the penetrating captive bolt is an effective method of euthanasia for use in slaughterhouses, in research facilities, and on the farm when use of drugs is inappropriate.

Disadvantages— (1) It is aesthetically displeasing. (2) Death may not occur if equipment is not maintained and used properly.

EUTHANASIA BY A BLOW TO THE HEAD

Euthanasia by a blow to the head must be evaluated in terms of the anatomic features of the species on which it is to be performed. The anatomic features of neonatal calves, however, make a blow to the head in this species unacceptable. • Personnel performing euthanasia by use of a blow to the head must be properly trained and monitored for proficiency with this method of euthanasia, and they must be aware of its aesthetic implications.

GUNSHOT

A properly placed gunshot can cause immediate insensibility and humane death. In some circumstances, a gunshot may be the only practical method of euthanasia.

Advantages—(1) Loss of consciousness is instantaneous if the projectile destroys most of the brain. (2) Given the need to minimize stress induced by handling and human contact, gunshot may at times be the most practical and logical method of euthanasia of wild or free-ranging species.

Disadvantages—(1) Gunshot may be dangerous to personnel. (2) It is aesthetically unpleasant. (3) Under field conditions, it may be difficult to hit the vital target area.

CERVICAL DISLOCATION

Cervical dislocation is a technique that has been used for many years and, when performed by well trained individuals, appears to be humane. However, there are few scientific studies to confirm this observation.

Advantages—(1) Cervical dislocation is a technique that may induce rapid loss of consciousness. (2) It does not chemically contaminate tissue. (3) It is rapidly accomplished.

Disadvantages—(1) cervical dislocation may be aesthetically displeasing to personnel. (2) Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced. (3) Its use is limited to poultry, other small birds, mice, and immature rats and rabbits.

DECAPITATION

Decapitation can be used to euthanatize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated.

Advantages—(1) Decapitation is a technique that appears to induce rapid loss of consciousness. (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished.

Disadvantages—(1) Handling and restraint required to perform this technique may be distressful to animals. (2) The interpretation of the presence of electrical activity in the brain following decapitation has created controversy and its importance may still be open to debate.

ELECTROCUTION:-

Electrocution, using alternating current, has been used as a method of euthanasia for species such as dogs, cattle, sheep, swine, foxes, and mink. Electrocution induces death by cardiac fibrillation, which causes cerebral hypoxia. However, animals do not lose consciousness for 10 to 30 seconds or more after onset of cardiac fibrillation.

Advantages—(1) Electrocution is humane if the animal is first rendered unconscious. (2) It does not chemically contaminate tissues. (3) It is economical.

Disadvantages—(1) Electrocution may be hazardous to personnel. (2) When conventional single-animal probes are used, it may not be a useful method for mass euthanasia because so much time is required per animal.

MICROWAVE IRRADIATION

Heating by microwave irradiation is used primarily by neurobiologists to fix brain metabolites in vivo while maintaining the anatomic integrity of the brain. Microwave instruments have been specifically designed for use in euthanasia of laboratory mice and rats.

Advantages (1) Loss of consciousness is achieved in less than 100ms, and death in less than 1 second. (2) This is the most effective method to fix brain tissue in vivo for subsequent assay of enzymatically labile chemicals.

Disadvantages—(1) Instruments are expensive. (2) Only animals the size of mice and rats can be euthanatized with commercial instruments that are currently available.

THORACIC (CARDIOPULMONARY, CARDIAC) COMPRESSION:

Thoracic (cardiopulmonary, cardiac) compression is used to euthanatize small- to medium-sized free ranging birds when alternate techniques described in these guidelines are not practical.

Advantages—(1) This technique is rapid. (2) It is apparently painless. (3) It maximizes carcass use for analytical/contaminant studies.

Disadvantages—(1) It may be considered aesthetically unpleasant by onlookers. (2) The degree of distress is unknown.

KILL TRAPS

Mechanical kill traps are used for the collection and killing of small, free-ranging mammals for commercial purposes (fur, skin, or meat), scientific purposes, to stop property damage, and to protect human safety.

Advantage—Free-ranging small mammals may be killed with minimal distress associated with handling and human contact.

Disadvantage: Traps may not afford death within acceptable time periods. (2) Selectivity and efficiency is dependent on the skill and proficiency of the operator.

MACERATION:

Maceration, via use of a specially designed mechanical apparatus having rotating blades or projections, causes immediate fragmentation and death of day-old poultry and embryonated eggs.

Advantages—(1) Death is almost instantaneous. (2)The method is safe for workers. (3) Large numbers of animals can be killed quickly.

Disadvantages—(1) Special equipment is required. (2) Macerated tissues may present biosecurity risks.

ADJUNCTIVE METHODS

Stunning and pithing, when properly done, induce loss of consciousness but do not ensure death. Therefore, these methods must be used only in conjunction with other procedures, such as pharmacologic agents, exsanguination, or decapitation to euthanatize the animal.

Exsanguination

Exsanguination can be used to ensure death subsequent to stunning, or in otherwise unconscious animals. Because anxiety is associated with extreme hypovolemia, exsanguination must not be used as a sole means of euthanasia. Animals may be exsanguinated to obtain blood products, but only when they are sedated, stunned, or anesthetized.

Stunning

Animals may be stunned by a blow to the head, by use of a non- penetrating captive bolt, or by use of electric current. Stunning must be followed immediately by a method that ensures death. • Blow to the head—Stunning by a blow to the head is used primarily in small laboratory animals with thin craniums. A single sharp blow must be delivered to the central skull bones with sufficient force to produce immediate depression of the central nervous system. When properly done, consciousness is lost rapidly.

Non-penetrating captive bolt

A non-penetrating captive bolt may be used to induce loss of consciousness in ruminants, horses, and swine. Signs of effective stunning by captive bolt are immediate collapse and a several second period of tetanic spasm, followed by slow hind limb movements of increasing frequency. Other aspects regarding use of the non-penetrating captive bolt are similar to the use of a penetrating captive bolt, as previously described.

Electrical stunning

Alternating electrical current has been used for stunning species such as dogs, cattle, sheep, goats, hogs, fish and chickens. Experiments with dogs have identified a need to direct the electrical current through the brain to induce rapid loss of consciousness. In dogs, when electricity passes only between fore- and hind limbs or neck and feet, it causes the heart to fibrillate but does not induce sudden loss of consciousness. For electrical stunning of any animal, an apparatus that applies electrodes to opposite sides of the head, or in another way directs electrical current immediately through the brain, is necessary to induce rapid loss of consciousness.

Pithing

In general, pithing is used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means. For some species, such as frogs, with anatomic features that facilitate easy access to the central nervous system, pithing may be used as a sole means of euthanasia, but an anesthetic overdose is a more suitable method.

Important Questions

Very Short Questions (2 marks each)

1. What does CPCSEA stand for?
2. Name two OECD guidelines for the maintenance of laboratory animals.
3. Define the term "laboratory animal."
4. List two species commonly used in laboratory experiments.
5. What is a transgenic animal?
6. Name two common routes of drug administration in laboratory animals.
7. What is euthanasia in the context of laboratory animals?
8. Define "knockout mouse."
9. Name one common site for blood collection in mice.
10. What is the primary use of zebrafish in laboratory research?

Short Questions (5 marks each)

1. Describe the main objectives of CPCSEA guidelines.
2. Explain the OECD guidelines for the breeding of laboratory animals.
3. Compare the use of mice and rats in laboratory experiments.
4. Discuss the ethical considerations in the use of transgenic animals.
5. Summarize the methods of euthanasia as per CPCSEA guidelines.
6. Describe the characteristics of BALB/c mice and their common uses in research.
7. Explain the applications of Sprague-Dawley rats in laboratory experiments.

8. Illustrate the procedure for cardiac puncture and its applications.
9. Compare the advantages of using inbred vs. outbred strains in research.
10. Discuss the role of anesthesia in blood collection and its impact on the results.

Long Questions (10 marks each)

1. Analyze the significance of CPCSEA and OECD guidelines in the context of laboratory animal research.
2. Evaluate the ethical implications of using transgenic and mutant animals in scientific research.
3. Critically assess the different routes of drug administration in laboratory animals, highlighting their advantages and disadvantages.
4. Describe the various techniques of blood collection from laboratory animals and their applications.
5. Discuss the role of laboratory animals in the development of new drugs and therapies.
6. Compare and contrast the applications and limitations of different species and strains of laboratory animals.
7. Analyze the ethical, scientific, and practical considerations in the use of non-human primates in experiments.
8. Evaluate the advancements in genetic engineering techniques and their impact on the development of transgenic and mutant animals.
9. Compare the guidelines provided by CPCSEA and OECD for blood collection and euthanasia in laboratory animals.
10. Discuss the ethical and regulatory challenges in the conduct of experiments on laboratory animals.