

Unit 1: Laboratory Animals

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Pharmacological and Toxicological Screening Methods I, subject code MPL103T, is a core Semester 1 subject of M.Pharm Pharmacology. It carries 4 credits and 60 hours of theory, taught at 4 hours per week, for 100 marks. Before any drug reaches a patient it must be screened: tested in living systems and in test systems that imitate living ones, to find out whether it works, how strongly it works, and where its dangers begin. This subject teaches that preclinical evaluation end to end. Unit 1 lays the foundation: the common laboratory animals and their strains, handling, transgenic animals, anaesthesia and euthanasia, animal house maintenance and breeding, the CPCSEA guidelines that govern animal experiments in India, good laboratory practice, and the principles and methods of bioassay. Units 2 to 5 then apply the idea of screening system by system. Unit 2 covers the general principles of preclinical screening and the screening of CNS and autonomic drugs, from behavioural tests to models of Parkinson's disease and Alzheimer's disease. Unit 3 covers respiratory, reproductive, analgesic, anti-inflammatory, antipyretic and gastrointestinal screening. Unit 4 covers cardiovascular screening, metabolic disorder screening, anticancer screening and hepatoprotective screening. Unit 5 covers immunomodulator screening, the principles and evaluation of immunoassay with the immunoassays of digoxin and insulin, the limitations of animal experimentation and its alternatives, and the extrapolation of in vitro and animal data towards humans. A student who completes this subject should be able to choose the right animal and the right model for a given drug class, name the endpoint and the standard drug of every classical screen, and judge what a preclinical result can and cannot promise for the clinic.

1.1 Why animals stand at the start of drug discovery

A new chemical cannot be given to a human being on the strength of its structure or its behaviour in a test tube. The whole animal adds what no isolated system can: absorption, distribution, metabolism and excretion acting together, intact reflexes and feedback loops, and the integrated response of organs that talk to each other. **Preclinical screening** is the organised testing of new substances in animals and in animal derived systems to detect pharmacological activity, estimate potency, and give the first warning of toxicity before human exposure. Every screen in this subject follows the same grammar: a defined animal or preparation, a reproducible stimulus or disease like state, a measurable **endpoint**, and comparison against a **standard drug** whose behaviour in that model is already known.

The choice of species is itself a scientific decision. The animal must respond to the drug class in a way that resembles the human response, it must be economical enough to use in groups large enough for statistics, and its use must be ethically justified. The sections below take the common species one by one.

1.2 The mouse: the workhorse of screening

The mouse (*Mus musculus*) is the most widely used laboratory animal, accounting for the majority of animals used in pharmacology worldwide. It is small (adult weight about 20 to 40 g), cheap to house and feed, breeds rapidly with a gestation of about 19 to 21 days and large litters, and its small size means that only milligram quantities of a scarce new compound are needed per animal, a decisive advantage in early screening.

Applications follow from these virtues. Mice are the standard species for acute toxicity observation, for analgesic screening (writhing, hot plate), for CNS screening (locomotor activity, convulsions), for cancer

chemotherapy screening using transplantable tumours, and for immunological work. Genetically defined mice, including transgenic and knockout lines (section 1.7), make the mouse the principal species for studying the role of a single gene or receptor in drug action. Its limitations are its very small blood volume, which restricts repeated sampling, and metabolic differences from humans that can mislead extrapolation (Unit 5).

1.3 The rat: the pharmacologist's standard animal

The rat (*Rattus norvegicus*) is the second great screening species and the default animal of experimental pharmacology teaching and research. Adults weigh about 200 to 400 g, large enough for surgery, repeated blood sampling and blood pressure recording, yet still economical. Two outbred stocks dominate: the **Wistar rat**, an albino stock valued for its docility, and the **Sprague Dawley rat**, a fast growing albino stock widely used in toxicology and reproduction studies.

Rats are the standard species for anti-inflammatory screening (carrageenan paw oedema), anti-ulcer studies (pylorus ligation), behavioural pharmacology (conditioned avoidance, maze learning), antihypertensive and diuretic screening, and most chronic toxicity work. The rat lacks a gall bladder and cannot vomit, two anatomical facts that shape its use: it is unsuitable for emetic studies, and bile collection is continuous rather than stored. Oestrous cycle monitoring by vaginal cytology makes the rat the standard species for reproductive screening (Unit 3).

1.4 Guinea pig, rabbit, hamster and the larger species

Each remaining species survives in the laboratory because it does one job better than the rodents.

Species	Special features	Classic applications
Guinea pig (<i>Cavia porcellus</i>)	Bronchial smooth muscle very sensitive to histamine; needs dietary vitamin C; ileum rich in histamine and acetylcholine receptors	Isolated ileum bioassay, bronchospasm and anti-asthmatic screening, anaphylaxis and allergy models, hypoxia studies less so
Rabbit (Or <i>Yctolagus cuniculus</i>)	Docile, large ear veins for intravenous dosing and sampling; eye accessible; some strains resistant to atropine	Pyrogen testing (now largely replaced), eye irritation and ocular pharmacology, isolated heart and ear preparations, pregnancy and teratology work
Syrian hamster	Cheek pouch accessible for study; hibernation physiology	Buccal pouch carcinogenesis, metabolic and infection models
Cat	Well developed vomiting centre; cardiovascular and CNS physiology close to classical teaching preparations	Emetic and anti-emetic work (historical), blood pressure and neurophysiology preparations
Dog (beagle)	Cardiovascular system and gastric physiology resemble humans; manageable size in the beagle	Cardiovascular safety and haemodynamic studies, emetic screening, chronic toxicology as the standard non-rodent species
Monkey (rhesus, <i>Cynomolgus</i>)	Closest physiology and genetics to humans; reserved for questions rodents cannot answer	Reproductive pharmacology, CNS and behavioural studies, biologics and vaccine evaluation, used sparingly on ethical grounds

The pattern to remember is that species selection is driven by the endpoint: the guinea pig ileum for spasmogens, the dog for emesis and haemodynamics, the rabbit for pyrogens and the eye, the primate only when no lower species can model the human system.

1.5 Strains: inbred, outbred and mutant

A **strain** is a population of animals with a defined genetic relationship, and the choice of strain decides the reproducibility of the whole experiment.

- **Outbred stocks** (Wistar, Sprague Dawley, Swiss albino mice) are bred to preserve genetic variation, so results generalise across genotypes much as a human population varies. They are cheaper and are the default for routine screening.
- **Inbred strains** are produced by brother to sister mating for at least 20 generations, making animals within the strain almost genetically identical, like identical twins. Examples are BALB/c and C57BL/6 mice and the Fischer 344 rat. Inbred strains remove genetic noise, accept tissue grafts from each other, and each strain has a characteristic profile (C57BL/6 mice, for example, are the background of most transgenic lines). Their weakness is that a result may be true only for that one genotype.
- **Mutant and specialised strains** carry a defined abnormality that is itself the model: the spontaneously hypertensive rat (SHR) for hypertension, the obese Zucker rat and the db/db mouse for metabolic disease, the nude (athymic) mouse that accepts human tumour xenografts, and the SCID mouse lacking functional immunity.

Sex, age and weight are part of the same discipline: a screening protocol states them for every group, because hormonal state, age and body weight all change drug response.

1.6 Handling and restraint

Correct handling is both an ethical duty and a scientific one, because a frightened, struggling animal releases catecholamines and corticosteroids that distort blood pressure, glucose, behaviour and almost every endpoint in this subject. The general rules are consistent across species: approach calmly, handle animals regularly before the experiment so they habituate, support the body weight fully, restrain for the shortest time needed, and never lift a rodent by the tip of the tail alone or squeeze the thorax.

In practice: mice are picked up by the base of the tail and restrained by scruffing the skin over the neck between finger and thumb, which also presents the abdomen for intraperitoneal injection. Rats are grasped firmly around the thorax from above with the head controlled, or scruffed for injections. Rabbits are lifted with one hand under the hindquarters and the ears and scruff controlled with the other, never by the ears alone. Guinea pigs are cupped with one hand under the chest and the other supporting the hindquarters. Restrainers (tubes and boxes sized to the animal) are used for dosing and sampling, and chemical restraint (section 1.9) replaces force whenever the procedure is more than brief.

Routes of administration taught with handling are oral (gavage tube in rodents), intraperitoneal, subcutaneous, intramuscular and intravenous (tail vein in rodents, ear vein in the rabbit, saphenous or jugular in larger animals).

1.7 Transgenic animals: production

A **transgenic animal** carries foreign DNA, or a deliberately altered version of its own DNA, in its genome, passed to its offspring through the germ line. Production follows three classical routes.

- 1 **Pronuclear microinjection:** the DNA construct is injected into one pronucleus of a fertilised egg, which is then implanted into a pseudopregnant foster mother. Some pups integrate the construct and are screened for its presence; founders are bred to establish a line. Integration is random, so expression varies between lines.
- 2 **Embryonic stem cell targeting:** the construct is introduced into embryonic stem cells in culture, where **homologous recombination** can place it at an exact locus. This precision allows **knockout** animals, in which a gene is disabled, and **knock-in** animals, in which a gene is replaced by a modified or human version. Selected stem cells are injected into a blastocyst, producing a chimeric animal whose germ line transmission gives the new strain.

- 3 **Viral and newer editing routes:** retroviral and lentiviral vectors carry constructs into embryos, and nuclease based editing (the CRISPR Cas9 system) cuts DNA at a chosen sequence so that genes can be disrupted or corrected directly in the zygote, greatly speeding production.

1.8 Transgenic animals: maintenance and applications

Transgenic lines are maintained as breeding colonies with genotyping of each generation (usually from a tail or ear sample), because the inserted gene may be present in one or two copies and expression can drift. Cryopreservation of embryos and sperm protects valuable lines against disease and genetic drift.

Applications in pharmacology are direct. **Target validation:** if deleting a receptor gene abolishes the effect of a drug class, the receptor is confirmed as the target (the principle behind many knockout studies of opioid, dopamine and cannabinoid receptors). **Disease models:** transgenic mice expressing human amyloid precursor protein mutations model Alzheimer's disease for the screening of Unit 2; mice carrying human oncogenes model cancer. **Humanised animals** expressing human drug metabolising enzymes or human receptors predict human responses better than the wild type animal. **Reporter animals**, in which a gene of interest drives a visible marker, turn gene expression into a readable endpoint.

1.9 Anaesthesia of experimental animals

Anaesthesia in screening serves the animal (freedom from pain and distress) and the experiment (immobility for surgery and recording). The agent is chosen by species, duration and the endpoint at stake, because most anaesthetics themselves depress the CNS, respiration and cardiovascular system and can distort results.

Agent or combination	Route and use	Notes
Ketamine with xylazine	Injectable, rodents and larger animals, short to medium procedures	Widely used combination; xylazine adds analgesia and muscle relaxation but depresses respiration and heart rate
Thiopentone and pentobarbitone	Injectable barbiturates, classical rodent and rabbit anaesthesia	Narrow margin between anaesthesia and respiratory death; pentobarbitone now more often an overdose agent for euthanasia
Isoflurane and sevoflurane	Inhalational, all species, precise control	Rapid induction and recovery, depth adjusted minute to minute; need a vaporiser and scavenging
Urethane	Injectable, terminal (non recovery) experiments only	Long lasting anaesthesia for acute cardiovascular and nerve recordings; carcinogenic, never used where the animal recovers
Local anaesthetics (lidocaine)	Infiltration or nerve block	For minor procedures, and combined with general agents to reduce their dose

Stages of anaesthesia, depth monitoring (pedal reflex, respiratory rate, heart rate), body temperature support and fluid care are part of competent practice: a hypothermic anaesthetised rodent produces blood pressure and metabolic data that belong to the cold, not to the drug.

1.10 Euthanasia of experimental animals

Euthanasia is the humane killing of an animal at the end of an experiment, or earlier when a humane endpoint is reached (unrelieved pain, severe weight loss, moribund state). CPCSEA accepts methods that produce rapid unconsciousness and death with minimal pain and distress, and the method must not destroy the tissue or endpoint being studied.

Accepted routes include overdose of an anaesthetic agent (barbiturate overdose is the commonest physical chemical method), inhalation of carbon dioxide in rodents with a gradual fill of the chamber, and, in deeply anaesthetised animals, physical methods such as cervical dislocation in small rodents performed by trained personnel. Death must be confirmed (absence of heartbeat and respiration, fixed pupils) before disposal. Neonates, which resist hypoxia, need special care and longer exposure or an injectable agent. The scientific point is the mirror of anaesthesia: the method of death can alter blood chemistry, stress hormones and tissue enzymes, so it is standardised and recorded like any other part of the protocol.

1.11 Maintenance of the animal house: housing and environment

Maintenance begins with the building. A laboratory animal facility is barrier managed: clean and dirty corridors, controlled entry, quarantine for incoming animals, and separation of species and of infected from clean stock. Standard conditions in Indian guidelines for rodents are a temperature of about 22 plus or minus 3 degrees Celsius, relative humidity of 30 to 70 percent, 12 hour light and dark cycles, and 10 to 15 fresh air changes per hour, with noise and vibration controlled.

Cage level care decides data quality. Cages are sized to species norms so animals can stand, turn and rest normally; bedding is absorbent, dust free and changed on schedule; feed is a standardised pelleted diet (so that nutrition does not vary between batches of an experiment) with clean drinking water ad libitum; and environmental enrichment (nesting material, shelters) reduces stress behaviour. Health monitoring is continuous: sentinel animals, microbiological screening and post mortem checks keep specific pathogen free colonies genuinely free, because a silent infection changes immune and metabolic endpoints across an entire facility. Every animal carries records: species, strain, sex, source, date of birth and experimental allocation.

1.12 Breeding of laboratory animals

Breeding systems protect the genetics described in section 1.5. **Monogamous pairing** (one male with one female, the standard for mice) allows exact pedigree records. **Polygamous or harem systems** (one male with several females) save space for production colonies. **Inbreeding** by brother to sister mating maintains inbred strains and must be tracked generation by generation; **outbreeding** uses rotation systems that avoid mating close relatives, preserving the variation that outbred stocks exist to provide.

Practical numbers shape colony planning: mice gestate in about 19 to 21 days with litters commonly of 6 to 12 pups and can be rebred at the post partum oestrus; rats gestate in about 21 to 23 days; rabbits in about 30 to 32 days; guinea pigs in about 59 to 72 days with small precocious litters. Weaning is at about 3 weeks in mice and rats. A breeding nucleus of genetically monitored parents supplies production animals, so that experimental animals are never bred casually from the experimental stock itself.

1.13 CPCSEA guidelines and the ethics framework

In India, animal experimentation is regulated under the Prevention of Cruelty to Animals Act, 1960, through the **Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)**, a statutory body under the Ministry of Fisheries, Animal Husbandry and Dairying. No institution may conduct animal experiments unless it is registered with CPCSEA, and no experiment may begin without approval of its protocol by the institution's own **Institutional Animal Ethics Committee (IAEC)**.

The framework, in sequence, is:

FROM IDEA TO APPROVED EXPERIMENT (CPCSEA / IAEC)

```
Researcher drafts protocol
(species, numbers, justification, humane endpoints)
|
v
IAEC review: CPCSEA nominee must be present
(welfare, 3Rs, statistics, alternatives considered)
|
approved? ---- no ----> revise and resubmit
| yes
v
Registered establishment, CPCSEA norms for housing,
breeding and transport; records kept and inspected
|
v
Experiment runs; humane endpoints enforced;
annual report and IAEC minutes to CPCSEA
```

The IAEC includes the CPCSEA nominee, a scientist from a different biological discipline, a veterinarian, and a socially aware member from outside the institution, so that welfare is judged by more than the experimenters themselves. CPCSEA norms also fix housing space, breeding and transport standards, and require that experiments use the minimum number of animals consistent with statistical validity, that alternatives be considered first (the 3Rs of Unit 5), and that anaesthesia and analgesia be used wherever pain is possible. Experiments on large animals and primates attract stricter scrutiny, and the CPCSEA main committee itself must clear certain categories.

CPCSEA and IAEC: From Protocol to Approved Experiment



Every protocol is judged against the 3Rs before approval.

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Figure: The CPCSEA and IAEC approval path. No animal experiment in India begins before the Institutional Animal Ethics Committee, with the CPCSEA nominee present, has approved the protocol.

1.14 Good laboratory practice

Good laboratory practice (GLP) is the quality system under which non clinical safety studies are planned, performed, monitored, recorded, archived and reported, so that a regulator can trust and reconstruct them years later. Its principles, harmonised through the OECD and monitored in India by the National GLP Compliance Monitoring Authority, include:

- A named **study director** responsible for the whole study, and an independent **quality assurance unit** that inspects it.

- Written **standard operating procedures** for every routine operation, and a study plan (protocol) approved before work begins.
- Characterised test items and test systems, calibrated instruments, and trained, documented personnel.
- **Raw data** recorded indelibly and contemporaneously; every correction traceable to a person and a reason.
- A final report that mirrors the raw data, with archives retained for the prescribed period.

In this subject GLP matters for two reasons: safety data submitted for drug approval must be GLP generated, and even academic screening borrows its disciplines (protocol first, raw data always, no unrecorded adjustments) because they are what make a result believable.

1.15 Bioassay: principle and scope

A **bioassay** (biological standardisation) measures the potency of a substance by the response it produces in a living system: an animal, an isolated tissue, a cell culture or an enzyme preparation. The **principle** is comparison: the unknown preparation is tested side by side with a **standard preparation** of the same substance (ideally an international or pharmacopoeial standard), and potency is expressed relative to that standard, never as an absolute property of the tissue.

Bioassay is the method of choice in defined circumstances, which form its **scope**:

- The active principle is unknown or cannot yet be measured chemically (historically insulin, posterior pituitary and many hormones and vitamins).
- No chemical method is sensitive or specific enough, or the substance exists as a mixture of related actives whose combined effect matters.
- The biological activity itself is the property of interest, as with vaccines, antisera and cytokines.
- A screening endpoint is needed for a new substance whose chemistry gives no clue to activity.

Its **limitations** are the reverse of its virtues: living systems vary, so bioassay is less precise than chemical assay and needs statistical design to reach acceptable error; it is slower, costlier and uses animals; and the response may not be specific, so interfering substances falsify results. Wherever a validated chemical or physical assay exists, pharmacopeias replace bioassay with it; bioassay survives where biology is the only measuring instrument available.

1.16 Bioassay: methods

Bioassay designs are classified by how the comparison with the standard is made.

Design	How it works	Typical example
Matching (bracketing)	The dose of unknown producing the same response as a fixed standard dose is found by trial, bracketing the response between standard doses	Early insulin and pituitary assays; simple but imprecise
Interpolation	The response to the unknown is read off a previously plotted standard dose response curve	Reading an unknown histamine concentration from the guinea pig ileum curve
Multiple point (quantal and graded) assays	Several doses of standard and unknown are compared in a designed experiment: 2+2, 3+3 or 4+4 dose assays, with parallel line statistics	Pharmacopoeial assays of insulin, digitalis, hormones
Quantal (end point) methods	The response is all or none (dead or alive, convulsed or not); the dose effective in 50 percent of animals (ED50) or lethal in 50 percent (LD50) is estimated	Convulsion protection assays, toxicity endpoints

Design	How it works	Typical example
Microbiological assays	Growth or inhibition of a microorganism measures the substance	Antibiotic potency by cylinder plate or turbidimetric methods

A 3 + 3 GRADED BIOASSAY, STEP BY STEP

Plot log dose response curves for standard (S1 S2 S3)
and test (T1 T2 T3) at three dose levels each

|

v

Check validity: curves linear and parallel
(non parallelism means the test is not behaving
like the standard; the assay is invalid)

|

v

Horizontal distance between the two lines
= log potency ratio; convert to potency units

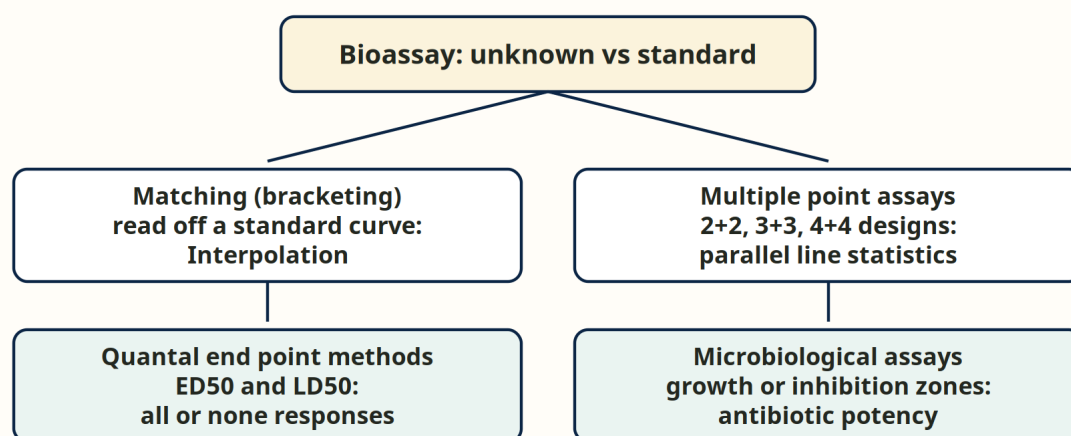
|

v

Report potency with fiducial (confidence) limits

Preparation choice follows the substance: isolated guinea pig ileum for histamine and acetylcholine like activity, isolated frog rectus abdominis historically for acetylcholine, rabbit blood sugar or mouse convulsion methods historically for insulin, rat blood pressure for catecholamines. Every valid assay ends in statistics: potency is reported with fiducial limits, because a bioassay number without its error is not a result.

Bioassay Designs: How Unknown Is Compared With Standard



Every valid assay reports potency with fiducial limits.

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Figure: The four families of bioassay design. All compare the unknown against a standard; they differ in how the comparison is arranged and analysed.

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