

PharmacoLearn

MPL103T Pharmacological and Toxicological Screening Methods I, M.Pharm Semester 1

Unit 2: Preclinical Screening: General Principles, CNS and Autonomic Pharmacology

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2.1 General principles of preclinical screening

Screening is organised as a funnel. A new substance enters at the wide end, where cheap, fast, high capacity tests remove the inactive majority, and leaves at the narrow end, where expensive, slow, whole animal studies characterise the few survivors.

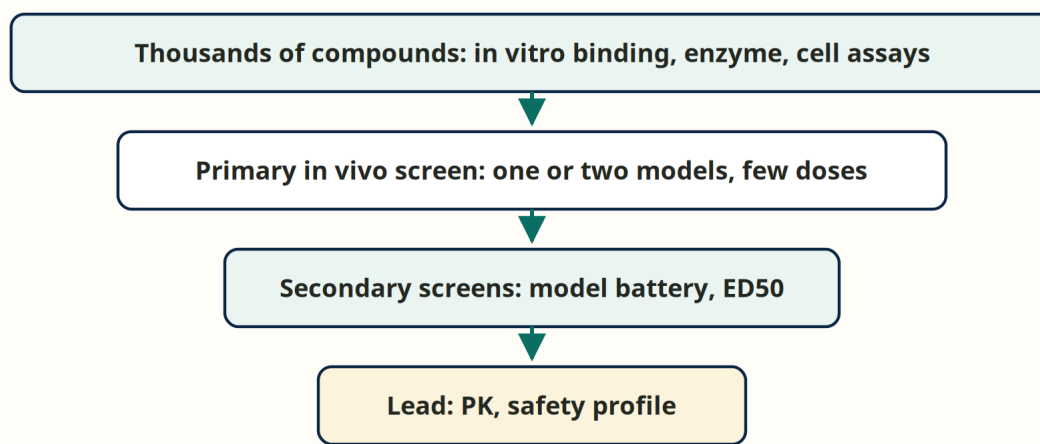
The principles that govern every unit of this subject are:

- **Start simple, proceed to the complex.** Primary screening in vitro (enzyme, receptor binding, cell systems) precedes secondary screening in vivo, which precedes detailed safety work.
- **Always run a standard.** Every experiment includes the reference drug of the class (diazepam in anxiety models, morphine in analgesia, haloperidol in psychosis models), so a failed day of testing is detected at once.
- **Use more than one model.** No single test defines a drug class; a profile across related models separates true activity from artefacts.
- **Quantify.** Dose response curves, ED50 values and potency relative to the standard replace impressions.
- **Blind and randomise.** The observer should not know which animal received which treatment, and animals are allocated randomly, because expectation moves subjective endpoints.
- **Screen for the unwanted early.** A compound active in the target model but toxic or sedating at the same dose is rejected early, using simple counter screens such as rotarod performance and general behavioural observation (the Irwin profile in mice, a systematic check of behaviour, autonomic signs and toxicity after a single dose).

THE SCREENING SEQUENCE

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Thousands of compounds
  | in vitro binding, enzyme, cell assays
  v
Primary in vivo screen (one or two models, few doses)
  | inactive majority discarded
  v
Secondary screens (model battery, dose response, ED50)
  | counter screens: motor, autonomic, toxicity
  v
Lead compound: pharmacokinetics, safety pharmacology
  |
  v
Candidate for formal toxicology and clinical trials
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The Preclinical Screening Cascade



Cheap fast tests first; expensive whole animal studies last.

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Figure: The screening funnel. Capacity falls and cost rises at every stage, so weak compounds must be removed as early as the science allows.

2.2 In vivo, in vitro and alternative models

The three families of test system trade realism against control.

System	What it is	Strengths	Weaknesses
In vivo	Whole living animal	Integrated ADME and organ interaction; the only system that shows behaviour, blood pressure and disease course together	Slow, costly, ethically loaded, species differences
In vitro	Isolated organs, tissues, cells, enzymes, receptors outside the body	Precise dose control, mechanism visible, high throughput, no whole animal suffering	No ADME, no integrated physiology; activity may vanish or appear falsely
Alternative models	Non animal or lower organism systems: cell lines, computer (in silico) models, zebrafish embryos, invertebrates	Replace or reduce animal use (Unit 5), fast and cheap	Predictive value must be proven model by model

Good programmes chain them: an in vitro hit earns an in vivo test; an in vivo profile earns comparison with human data before any clinical step.

2.3 Behavioural screening and locomotor activity

Behaviour is the readout of CNS screening, and the first question asked of any CNS active compound is whether it stimulates or depresses the intact animal.

- **Actophotometer:** the mouse or rat crosses beams of light in a cage; the count of crossings in a fixed time measures locomotor activity. Amphetamine raises the count, chlorpromazine and other sedatives lower it. It is the primary screen separating CNS stimulants from depressants.
- **Open field test:** the animal explores a novel arena; crossings, rearing and grooming are scored, and entries into the exposed centre reflect anxiety as much as movement.
- **Irwin primary observation:** a structured checklist (alertness, posture, gait, reflexes, autonomic signs, convulsions, death) after graded doses, giving the first behavioural and safety fingerprint of a new substance.

- **Hole board and exploratory tests:** head dipping into holes measures exploration, reduced by sedation and increased disinhibition by anxiolytics.

2.4 Muscle coordination and motor function

Motor tests serve twice: as screens in their own right (muscle relaxants, antiparkinsonian drugs) and as counter screens, because a drug that merely paralyses or sedates will falsely appear active in pain, anxiety and psychosis models.

- **Rotarod:** the animal walks on a rotating rod; the time it stays on measures coordination and balance. Neuroleptics, sedatives and muscle relaxants shorten the time; it defines the motor toxic dose (TD50) that is compared with the effective dose.
- **Grip strength meter:** measures forelimb grip force, reduced by muscle relaxants and neuromuscular blockers.
- **Traction test:** the mouse must pull itself up by the forepaws onto a wire; failure signals muscle relaxation.
- **Chimney test:** the mouse must climb backwards out of a vertical tube within a time limit; sedation and relaxation prolong it.
- **Inclined plane and screen tests:** the animal must hold position on a slope or mesh, a simple index of muscle tone and strength.

2.5 Screening of CNS stimulants

CNS stimulants are screened by reversal and enhancement designs. Increased locomotor counts in the actophotometer and increased open field activity are the primary signals. **Analeptic activity** (respiratory stimulation in barbiturate depressed animals) was the classical screen for agents like doxapram. Convulsant liability is measured by the dose lowering the threshold to electrically or chemically induced seizures, which is also a safety flag. Amphetamine serves as the standard stimulant; caffeine is the mild reference. A compound that increases activity only at doses that also cause stereotyped behaviour (repetitive sniffing, gnawing) is flagged as amphetamine like, with abuse potential implications.

2.6 Screening of CNS depressants

Depressants are detected by a coherent battery rather than one test: reduced actophotometer counts, **potentiation of barbiturate sleeping time** (the test drug prolongs the sleep produced by a fixed dose of pentobarbitone, the classical screen for sedative and hypnotic activity), loss of righting reflex at high doses, and reduced response to nociceptive and startle stimuli. Ethanol interaction and potentiation of ethanol induced sleep flag sedative load. Diazepam and pentobarbitone are the usual standards. The battery also grades depth: a compound that reduces activity but never abolishes the righting reflex is a sedative; one that abolishes it is a hypnotic or general depressant.

2.7 Screening of anxiolytics

Anxiety models exploit approach avoidance conflict: the animal wants to explore but fears exposure, light or punishment, and a true anxiolytic releases the suppressed behaviour without sedation.

Model	Principle	Endpoint	Standard
Elevated plus maze	Rodent avoids open, raised arms	Increased time and entries in open arms	Diazepam

Model	Principle	Endpoint	Standard
Light and dark box	Animal avoids the brightly lit compartment	More time and transitions into the light box	Diazepam
Vogel conflict test	Thirsty rat is punished by shock for drinking	Increased punished drinking (licks)	Diazepam
Four plate test	Mouse crossing between plates receives a shock	Increased punished crossings	Diazepam, buspirone
Social interaction test	Pairs of rats interact less under bright, unfamiliar conditions	Restored interaction time	Diazepam

The discipline of the battery is the rotarod and actophotometer run alongside: an apparent anxiolytic effect at a dose that also impairs coordination is sedation, not anxiolysis.

2.8 Screening of antipsychotics

Antipsychotic screening rests on dopamine blockade signatures.

- **Catalepsy test:** the drug treated animal maintains an imposed abnormal posture (forepaws on a raised bar) for an abnormally long time. Haloperidol and other D2 blockers produce catalepsy; it models the extrapyramidal liability of typical agents as much as their efficacy.
- **Blockade of amphetamine or apomorphine effects:** antipsychotics antagonise amphetamine induced hyperactivity and stereotypy, and apomorphine induced climbing in mice and stereotyped behaviour in rats.
- **Conditioned avoidance response:** trained rats avoid a shock on a warning signal; antipsychotics selectively suppress avoidance while leaving escape intact, one of the most predictive classical screens.
- **Pole climbing test:** the animal climbs a pole to escape shock; blockade of climbing parallels antipsychotic potency.

Atypical agents (clozapine, risperidone) are distinguished by weak catalepsy at doses that still block apomorphine climbing, the signature of a better extrapyramidal profile.

2.9 Screening of antiepileptics

Two complementary seizure models divide the drug class between them, and every new antiepileptic is run through both.

- **Maximal electroshock (MES):** a high current through corneal or ear electrodes produces tonic hindlimb extension in mice or rats. Abolition of the extensor phase signals activity against generalised tonic clonic seizures; phenytoin and carbamazepine are the standards.
- **Pentylenetetrazol (PTZ, metrazol) test:** a convulsant dose of PTZ produces clonic seizures; protection signals activity against absence and myoclonic seizures; ethosuximide and valproate are active here, phenytoin is not, which is exactly the clinical pattern.
- Supporting models include subcutaneous PTZ threshold testing, kindling (repeated subthreshold stimulation producing a chronic epileptic focus, for disease modification), and genetic absence models such as the GAERS rat.

The MES to PTZ pattern is the classic teaching point: matching the animal profile to the clinical seizure type is how screening predicts use.

Antiepileptic Screening: MES and PTZ Divide the Class

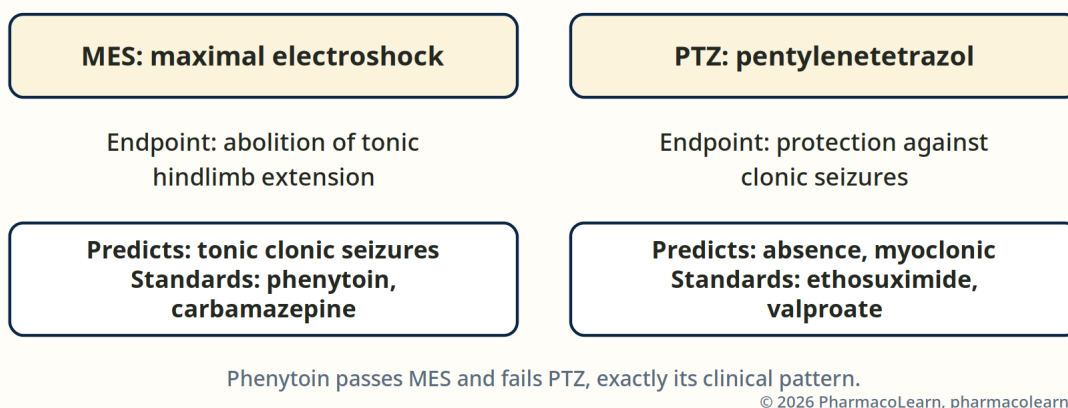


Figure: MES and PTZ divide the antiepileptic class between them; a new agent is always run through both.

2.10 Screening of nootropics and cognitive enhancers

Nootropics are screened in learning and memory models, usually against a pharmacologically induced amnesia.

- **Morris water maze:** the rat learns to find a hidden platform in a pool of opaque water using room cues; escape latency over training days measures spatial learning, and a probe trial without the platform measures memory.
- **Passive avoidance:** the animal learns to avoid a compartment where it received a shock; longer latency to re-enter on the test day means better memory. Scopolamine induced amnesia is the standard deficit, reversed by piracetam (the class standard) and by cholinesterase inhibitors.
- **Radial arm maze and Y maze:** working and reference memory are scored from arm choices, including spontaneous alternation in the Y maze, reduced by scopolamine and restored by nootropics.
- **Elevated plus maze transfer latency** and novel object recognition give simpler memory indices.

The trap of this area is stimulation masquerading as cognition: locomotor controls must accompany every memory claim.

2.11 Screening models of Parkinson's disease

Parkinson's models destroy or block the nigrostriatal dopamine pathway and ask whether the test drug restores movement.

- **Reserpine model:** reserpine depletes dopamine and produces akinesia, rigidity and hypothermia in rodents; levodopa and dopamine agonists reverse the syndrome.
- **Neurotoxin models:** 6-hydroxydopamine injected into the substantia nigra or striatum kills dopaminergic neurons on one side, producing rotational behaviour when the animal is challenged with apomorphine or amphetamine; the rotation count quantifies the lesion and its treatment. MPTP, which destroys nigral neurons through its metabolite MPP⁺, is used in mice and primates and is the closest toxin mimic of the human disease.
- **Haloperidol catalepsy** serves as a quick functional model of dopamine blockade.
- **Tremor models** (oxotremorine induced tremor) screen drugs for the tremor component, including anticholinergics such as trihexyphenidyl.

Levodopa with a peripheral decarboxylase inhibitor and bromocriptine are the reference standards.

2.12 Screening models of Alzheimer's disease and multiple sclerosis

Alzheimer's disease is modelled by deficit rather than by the full pathology. Scopolamine induced amnesia (section 2.10) screens symptomatic cholinergic drugs such as donepezil. Lesions of the basal forebrain cholinergic nuclei, intracerebral amyloid beta injection, and aged animals give deeper models. Transgenic mice overexpressing mutant human amyloid precursor protein develop amyloid plaques and memory loss and are the standard hosts for disease modifying candidates, including anti amyloid antibodies. Endpoints are maze performance plus amyloid and tau burden in brain tissue.

Multiple sclerosis is modelled by experimental autoimmune encephalomyelitis (EAE): rodents or primates are immunised with myelin proteins such as myelin basic protein or myelin oligodendrocyte glycoprotein in adjuvant, and develop ascending paralysis scored on a clinical scale. Fingolimod, glatiramer acetate and corticosteroids are active standards. Cuprizone feeding, which strips myelin chemically, models demyelination and remyelination. These are immunological models, linking forward to the immunomodulator screens of Unit 5.

2.13 Screening of drugs acting on the autonomic nervous system

Autonomic screening is the oldest branch of pharmacology and still runs on classical preparations.

- **Isolated tissues:** guinea pig ileum (acetylcholine, histamine), rat uterus, rabbit jejunum, rat vas deferens and the frog or rat rectus preparations quantify agonist potency and antagonism, with pA₂ values from Schild analysis (Unit 1 bioassay methods applied to receptors).
- **Whole animal haemodynamics:** blood pressure and heart rate in anaesthetised or conscious rats and dogs reveal sympathomimetic and sympatholytic action; the classical dog and cat blood pressure preparations defined adrenaline reversal and ganglion blockade.
- **Sympathomimetics** are profiled by pressor responses, mydriasis and bronchodilation; **sympatholytics** by blockade of those responses and by ptosis and sedation signals in rodents.
- **Parasympathomimetics and anticholinergics** are read from salivation, pupil size, gut motility (charcoal meal transit in mice), and protection against acetylcholinesterase inhibitor toxicity; atropine is the universal anticholinergic standard.
- **Neuromuscular blockers** are screened on the rat phrenic nerve diaphragm and chick biventer cervicis preparations and in vivo by grip strength and head drop in rabbits, separating depolarising (succinylcholine) from non depolarising (tubocurarine) patterns.

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