PharmacoLearn

MPL202T Pharmacological and Toxicological Screening Methods II, M.Pharm Semester 2

Unit 5: Toxicokinetics and Alternative Methods to Animal Toxicity Testing

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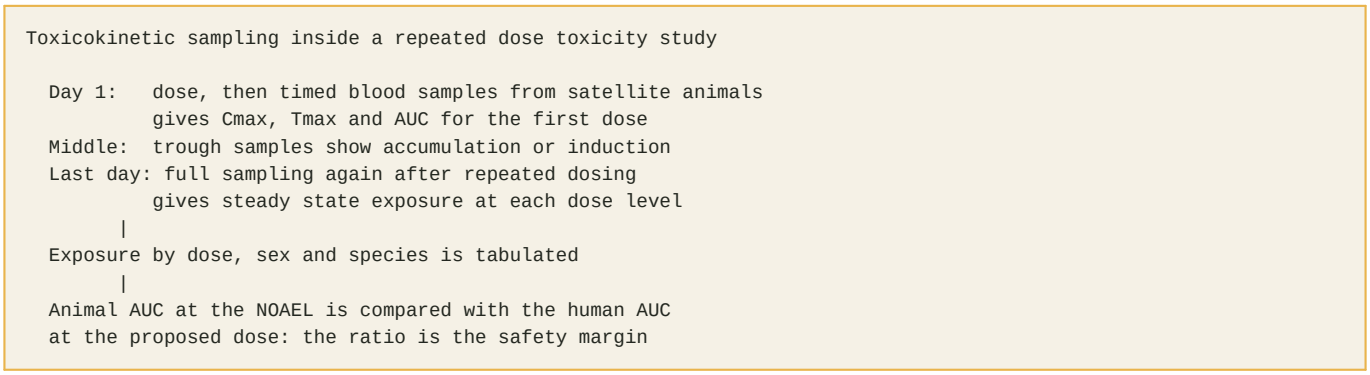
5.1 Toxicokinetics: measuring exposure inside the toxicity study

Pharmacokinetics asks what exposure a dose produces in a patient. **Toxicokinetics**, its twin under ICH S3A, asks the same question inside a toxicity study: what systemic exposure, measured as the area under the plasma concentration time curve (AUC) and the peak concentration (Cmax), did the animals actually experience at each dose level. The distinction between dose and exposure is the whole point. Two species given the same mg/kg dose may absorb, metabolise and clear the drug so differently that their blood exposures differ tenfold; comparing their toxicity by dose is then meaningless, while comparing by exposure is science.

5.2 Toxicokinetic evaluation in preclinical studies

In practice, toxicokinetic evaluation is woven into the studies of Unit 2 rather than run separately. Blood samples are taken from the main study animals where the species is large enough, or from dedicated **satellite groups** in rodent studies, so that bleeding does not distort the toxicity endpoints. Sampling is scheduled on the first day and near the end of dosing to detect change over time, and the assay must be a validated bioanalytical method for the drug (and major metabolites where relevant) in that species' plasma. The report expresses exposure per dose, per sex and per species, notes accumulation or its absence, and flags nonlinearity: exposure rising more, or less, than in proportion to dose.



5.3 Saturation kinetics: when more dose stops meaning more exposure

Saturation kinetics describes what happens when a process of absorption, metabolism or excretion reaches its maximum rate and cannot go faster. Below saturation, exposure rises in proportion to dose (first order behaviour). Above it, the clearing enzyme or transporter is working flat out (zero order behaviour), so each further increment of dose produces a disproportionate rise in blood concentration and a lengthening half life. The toxicological consequences are direct and heavily examined:

- A NOAEL set in the saturated region may not scale: small dose increases above it can produce large exposure jumps.
- High dose groups in a toxicity study may all sit at the same saturated exposure, collapsing the dose response the study was designed to measure.

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- Species differ in capacity, so the dose at which saturation occurs differs, one more reason dose comparisons mislead and exposure comparisons do not.

Toxicokinetic data are how saturation is detected: if doubling the dose quadruples the AUC, the study is in saturated territory and the interpretation must say so.

5.4 Importance and applications of toxicokinetic studies

The applications follow from the exposure principle. Toxicokinetics **interprets** the toxicity study: a negative study is only reassuring if exposure demonstrably exceeded the human exposure, and a positive finding is quantified against it. It builds the **safety margin**: the ratio of animal AUC at the NOAEL to the predicted human AUC at the therapeutic dose is the number on which first in human starting doses and dose escalation are argued. It guides **dose selection** for the longer studies, exposing saturation and accumulation before a two year study is locked into useless dose levels. It explains **species differences** and helps choose the most human relevant species for the definitive studies. And it satisfies a formal regulatory expectation under ICH S3A and M3(R2) that exposure data accompany the pivotal toxicity studies in an IND and marketing dossier.

5.5 Why alternative methods are needed

The final topic of the syllabus looks beyond the animal studies it has taught. Pressure to replace them comes from three directions at once: ethics, expressed since 1959 in the **3Rs** of replacement, reduction and refinement; science, because animal results predict human toxicity imperfectly and some human specific injuries are missed by every animal species; and regulation and economics, because validated alternatives are faster and cheaper and are increasingly written into law and guideline, with some sectors, such as cosmetics in the European Union, under full animal testing bans. Unit 5 of Screening Methods I introduced the 3Rs and extrapolation; this unit completes the picture at the level of toxicity testing itself.

5.6 In vitro and in silico alternatives

Replacement has advanced furthest exactly where the endpoint is local or cellular:

- **Skin corrosion and irritation**: reconstructed human epidermis models (OECD TG 431 and 439) now classify substances without rabbits.
- **Eye irritation**: the bovine corneal opacity and permeability test (TG 437), the isolated chicken eye test (TG 438) and reconstructed corneal epithelium models (TG 492) cover much of the TG 405 territory in defined approaches.
- **Genotoxicity**: the Ames test and the in vitro chromosomal assays of Unit 3 are themselves replacements of an earlier animal era, and cell based methods continue to grow.
- **Skin sensitization**: the adverse outcome pathway for sensitization has been broken into chemical and cell based key event assays (TG 442C, 442D and 442E), combined in defined approaches that can classify without the guinea pig or the mouse.
- **In silico methods**: (quantitative) structure activity relationship models, expert rule based systems and read across from tested analogues estimate toxicity from structure, and are accepted for some endpoints, notably the assessment of mutagenic impurities under ICH M7.

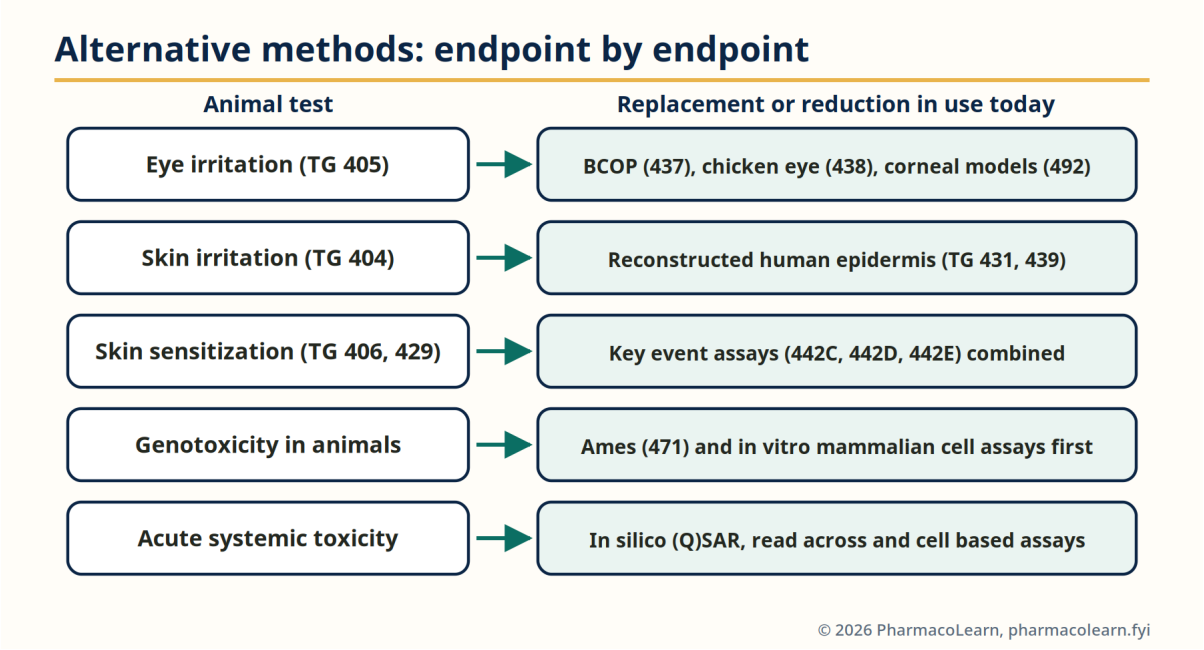

Figure: Replacement advances endpoint by endpoint: the local tolerance tests have largely fallen, and systemic endpoints are following.

5.7 What alternatives cannot yet do, and the road ahead

No in vitro system yet reproduces the integrated whole animal: absorption, metabolism, immune interaction and months of dosing across communicating organs. Chronic toxicity, carcinogenicity and reproductive toxicity therefore remain animal based at present, and regulators accept alternatives endpoint by endpoint, only after formal validation shows the new method predicts the old one's result (and, increasingly, the human result) reliably. The direction of travel is nevertheless unmistakable: integrated approaches to testing and assessment combine in silico predictions, in vitro key event data and targeted in vivo work into weight of evidence conclusions, and each validated replacement permanently retires an animal test. The student should leave this subject able to run the classical system and to see clearly where it is going.

Frequently Asked Questions

Q: What is the difference between MPL202T and MPL103T? A: MPL103T (Semester 1) teaches pharmacological screening: laboratory animals, bioassay, and the efficacy models used to show that a drug works. MPL202T (Semester 2) teaches toxicological screening: the regulatory toxicity studies, under OECD guidelines and GLP, that show whether a drug is safe enough to test in humans, ending with the IND enabling package, safety pharmacology and toxicokinetics.

Q: What are the four types of toxicology named in the PCI syllabus? A: General toxicology, which profiles the overall adverse effects of a substance; mechanistic toxicology, which explains how the injury happens at the molecular level; regulatory toxicology, which designs and judges studies to satisfy licensing authorities; and descriptive toxicology, which describes toxicity in practical terms for existing substances, poisoning treatment and hazard communication.

Q: Which OECD guidelines cover acute oral toxicity, and how do they differ? A: TG 420 (Fixed Dose Procedure) classifies by evident toxicity at fixed doses of 5, 50, 300 and 2000 mg/kg without using death as the endpoint; TG 423 (Acute Toxic Class Method) steps groups of three animals through the same fixed doses to place the substance in a hazard class; TG 425 (Up and Down Procedure) doses single animals sequentially with doses adjusted by a constant factor to estimate an LD50 with few animals. The classical LD50 guideline, TG 401, was deleted.

Q: What is the difference between sub-acute, sub-chronic and chronic toxicity studies? A: In the OECD framework used by the syllabus: sub-acute studies are the 28 day repeated dose studies (TG 407 oral, 410 dermal, 412 inhalation); sub-chronic studies are the 90 day studies (TG 408 oral in rodents, 409 in the non-rodent, 411 dermal, 413 inhalation); and chronic studies run for about 12 months or longer (TG 452), with TG 453 combining chronic toxicity and carcinogenicity in one design.

Q: Why is test item characterization compulsory in a regulatory toxicity study? A: Because the study compares groups that are meant to differ only in the dose of one defined substance. Characterization proves the identity and purity of that substance, its stability in storage and in the dosing vehicle, and, through dose formulation analysis, that each group actually received its intended dose. Without it, a toxicity finding cannot be attributed to the drug with confidence, and the study fails GLP expectations.

Q: What are segments I, II and III in reproductive toxicology? A: Segment I studies fertility and early embryonic development, dosing both sexes before and during mating and reading conception and implantation. Segment II is the teratogenicity or embryofoetal development study, dosing through organogenesis and examining foetuses for malformations, classically in rat and rabbit. Segment III studies pre and postnatal development, dosing the mother through lactation and following the offspring's survival, growth, development and reproductive capacity.

Q: How does the Ames test work, and why is S9 included? A: The Ames test (TG 471) exposes histidine dependent Salmonella typhimurium strains (with E. coli strains in the standard set) to the substance on minimal medium; only bacteria that undergo reverse mutation regain the ability to make histidine and form colonies, so colonies count mutations. The S9 rat liver fraction is included in parallel incubations because many mutagens are created by metabolism, and the parent substance alone would test negative.

Q: What studies are needed for an IND submission? A: The standard package contains the primary pharmacology and dose ranging data, the safety pharmacology core battery, the genotoxicity battery (Ames, an in vitro mammalian test and an in vivo test), repeated dose toxicity studies in a rodent and a non-rodent species of duration supporting the planned trial, toxicokinetic exposure data, and local tolerance data for the clinical route. Reproductive toxicity and carcinogenicity studies are staged later in the programme under ICH M3(R2).

Q: What is the hERG assay and why does it matter? A: The hERG assay measures block of the hERG potassium channel, which carries the IKr current that repolarises the ventricles. Block delays repolarisation, prolongs the QT interval and can cause torsades de pointes, the mechanism behind the withdrawal of drugs such as terfenadine and cisapride. Together with an in vivo QT study, under ICH S7B, it judges cardiac electrical risk before and during clinical development.

Q: What is saturation kinetics and why does it matter in toxicology? A: Saturation kinetics occurs when a metabolic or transport process reaches its maximum rate, so exposure stops rising in proportion to dose and instead climbs disproportionately, with a lengthening half life. It matters because dose based comparisons between species and dose levels break down in the saturated region, NOAEL interpretation changes, and only the exposure data from toxicokinetics reveal that the study has entered it.

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