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Unit-5

Toxicokinetics- Toxicokinetic evaluation in preclinical studies,

saturation kinetics Importance and applications of toxicokinetic studies.

Alternative methods to animal toxicity testing.

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Toxicokinetic

Toxicokinetics [TK] is the study of how a toxic substance (xenobiotic, drug, chemical, or poison) is absorbed, distributed, metabolised, and excreted (ADME) in a living organism.

Purpose:

To understand the fate of a toxin inside the body.

To determine the relationship between dose and systemic exposure.

To provide data for toxicological risk assessment and safe dose prediction.

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Key Processes (ADME in toxicology):-

Absorption – how the toxin enters the body (oral, inhalation, dermal, injection).

Distribution – how the toxin spreads into different tissues/organs via blood.

Metabolism (biotransformation) – how the body's enzymes (mainly in liver) chemically modify the toxin, sometimes detoxifying it or making it more toxic (bioactivation).

Excretion – removal of toxins or their metabolites, mainly via urine, bile, feces, sweat, or breath.

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Importance:

Helps in risk assessment of chemicals and drugs.

Supports dose-setting in animal studies and extrapolation to humans.

Identifies potential for bioaccumulation or long-term toxicity.

Explains differences in toxicity based on species, age, genetics, and exposure route.

TK evaluation = understanding "how much of the test substance reaches the body and causes toxicity" before human studies.

Objectives of TK Evaluation-

To confirm that animals are actually exposed to the test substance.

To study the relationship between dose, exposure, and toxicity.

To find safe dose levels and calculate safety margins (e.g., NOAEL).

To compare how different species (rat, dog, monkey, etc.) handle the substance.

To provide data required by regulatory agencies (ICH, OECD, FDA, EMA).

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Importance in Preclinical Studies-

Explains toxic effects based on exposure.

Helps in selecting safe starting doses for first-in-human trials.

Identifies if the substance accumulates or causes delayed toxicity.

Improves the relevance of animal data for humans.

When it is Done-

Along with acute (single-dose) studies.
In repeat-dose studies (14-day, 28-day, 90-day, chronic).
In both rodent species (rat, mouse) and non-rodents (dog, monkey).

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Parameters Measured-

Cmax - highest concentration in blood/plasma.

Tmax - time taken to reach Cmax.

AUC (Area Under Curve) - total exposure.

t½ (half-life) - time to reduce drug/toxin level by half.

Clearance (CL) - removal rate from plasma.

Volume of distribution (Vd) - extent of tissue distribution.

Accumulation ratio - buildup after repeated dosing.

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Methods Used-

Sample collection - blood, plasma, urine, bile, tissues.

Bioanalysis - HPLC or LC-MS/MS to measure drug/toxin levels.

Data analysis -
Non-compartmental analysis (direct calculation).
Compartmental modeling (mathematical models).

Methods of Toxicokinetic Evaluation

Step 1: Selection of Animals-

Usually two species:

One rodent (rat or mouse).
One non-rodent (dog or monkey).
Animals are the same as those used in toxicity studies.

Step 2: Dosing-

Test substance is given by the same route and dose as in toxicity studies (oral, IV, inhalation, dermal).
Can be single dose or repeat dose depending on the study design.

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Step 3: Sample Collection-

Blood/plasma samples collected at different time intervals.

Sometimes urine, bile, or tissue samples are also collected.

Sampling schedule is designed to cover absorption, distribution, metabolism, and excretion phases.

Step 4: Bioanalysis-

Samples are analysed using validated analytical methods like:

HPLC (High Performance Liquid Chromatography).

(Liquid Chromatography–Mass Spectrometry).

This measures the concentration of test substance (or its metabolites) in plasma/tissues.

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Step 5: Data Analysis

Concentration vs. time data is plotted.

Pharmacokinetic/Toxicokinetic parameters are calculated:

Cmax, Tmax, AUC, t½, Clearance, Volume of distribution, Accumulation ratio.

Analysis can be:

Non-compartmental analysis (NCA) → direct calculation from data.

Compartmental modelling → mathematical modelling of ADME.

Step 6: Interpretation

Exposure levels (e.g., AUC, Cmax) are compared with toxicological findings in animals.

This helps to establish:

Dose-exposure-toxicity relationship.

Species differences in metabolism/elimination.

Safety margins for human dose prediction.

Saturation Kinetics-

1. Definition

Saturation kinetics (also called non-linear kinetics or dose-dependent kinetics) occurs when the rate of drug metabolism or elimination does not increase proportionally with the drug concentration.

This happens because the enzymes or transport systems responsible for metabolism/elimination become saturated at higher drug concentrations.

Explanation

At low drug concentrations → enzymes are not saturated → elimination follows first-order kinetics (rate depends on concentration).

At high drug concentrations → enzymes get saturated → elimination follows zero-order kinetics (rate becomes constant, independent of concentration).
This results in non-linear pharmacokinetics.

Key Characteristics-

Rate of elimination becomes constant once saturation occurs.
Half-life ($t_{1/2}$) is not constant – it increases with dose.
Small increases in dose may cause disproportionately large increases in plasma concentration.
Risk of drug accumulation and toxicity is higher.

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Examples of Drugs Showing Saturation Kinetics-

Phenytoin (anticonvulsant).

Ethanol (alcohol).

Aspirin (at high doses).

Theophylline (sometimes at toxic levels).

Clinical Importance-

Drugs with saturation kinetics need careful dose adjustment.

Monitoring plasma levels is often required (e.g., phenytoin).

Overdose is more likely because elimination cannot keep up once enzymes are saturated.

Importance and Applications of Toxicokinetic Studies

1. Importance-

Toxicokinetic studies are important because they:

-Confirm systemic exposure

Ensure that test animals in toxicity studies are actually exposed to the drug/chemical.

-Link exposure with toxicity

Help to explain toxicological findings by comparing dose → exposure → toxic effect.

-Establish safety margins

Determine No Observed Adverse Effect Level (NOAEL) and support calculation of safe starting doses in humans.

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-Understand species differences

Compare how rodents, non-rodents, and humans metabolise and eliminate the test substance.

-Predict accumulation and long-term effects

Identify potential for bioaccumulation, persistence, or delayed toxicity.

-Regulatory requirement

International guidelines (ICH, OECD, FDA, EMA) mandate TK data in toxicity studies to support drug approval and chemical risk assessment.

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-Applications

Toxicokinetic studies are applied in:

Drug development

To set safe starting doses for first-in-human clinical trials.

To understand dose escalation and prevent toxicity.

Risk assessment of chemicals

Used in evaluating safety of pesticides, industrial chemicals, food additives, cosmetics.

Interpretation of toxicity studies

Relates exposure levels (Cmax, AUC) with observed toxic signs in animals.

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Species extrapolation

Helps in selecting the most suitable animal models for predicting human toxicity.

Regulatory submissions

Required for Investigational New Drug (IND) applications and safety dossiers.

Special cases

Useful in identifying non-linear (saturation) kinetics, drug-drug interactions, and accumulation at repeated doses.

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Introduction-

Traditional toxicity testing uses animals (rats, mice, rabbits, dogs, monkeys) to evaluate safety of drugs, chemicals, and cosmetics.

However, animal testing has ethical issues, high cost, long duration, and species differences that may not always predict human safety.

Therefore, alternative methods are being developed to reduce, refine, or replace animal use (3Rs principle – Replacement, Reduction, Refinement).

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Alternative Methods

(A) In vitro Methods (cell-based)

Use cultured human or animal cells/tissues to test toxicity.

Examples:

Cytotoxicity assays – test cell viability (e.g., MTT assay).

Human cell lines (liver, kidney, heart cells) for organ-specific toxicity.

3D cell cultures and organoids – mimic real human tissues.

Skin models – e.g., EpiDerm™, reconstructed human epidermis for skin irritation/corrosion testing.

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(B) In silico Methods (computer-based)

Use computer models and simulations to predict toxicity.

Examples:

QSAR (Quantitative Structure–Activity Relationship) models → predict toxicity based on chemical structure.

Molecular docking and simulations for interaction with biological targets.

ADME/toxicity prediction software.

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(C) Ex vivo Methods-

Use tissues or organs taken from animals/humans but studied outside the body.

Examples:

Isolated perfused liver or kidney models → metabolism and excretion studies.

Corneal tissues (bovine/human) → for eye irritation tests instead of Draize test.

(D) Organ-on-a-Chip Technology-

Microfluidic devices containing living human cells that mimic the structure and function of human organs (e.g., liver-on-a-chip, lung-on-a-chip).

Provide more accurate human-relevant data compared to animals.

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5. Stem Cell-based Assays-

Human induced pluripotent stem cells (iPSCs) or embryonic stem cells used.

Can differentiate into heart, liver, brain, kidney cells.

Useful for developmental toxicity, neurotoxicity, cardiotoxicity studies.

6. Microdosing and Human Volunteer Studies

Microdosing (Phase 0 studies): Very small, subtherapeutic doses given to humans.

Helps study pharmacokinetics and metabolism without significant risk.

Reduces dependency on animal models.

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Advantages of Alternatives

Ethically acceptable (reduces animal suffering)

More human-relevant data

Faster and cheaper

Supports the 3Rs principle (Replace, Reduce, Refine)

Limitations

Cannot fully replicate complex whole-body interactions.

Some regulatory bodies still require animal data for confirmation.