DEPTH OF BIOLOGY

Unit-2

Acute, sub-acute and chronic- oral, dermal and inhalational studies as per OECD guidelines.

Acute eye irritation, skin sensitization, dermal irritation & dermal toxicity studies.

Test item characterization- importance and methods in regulatory toxicology studies

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Acute, sub-acute and chronic- oral, dermal and inhalational studies as per OECD guidelines.

Toxicity studies are carried out to determine the safety profile of chemicals, drugs, and industrial compounds.

The Organisation for Economic Co-operation and Development (OECD) develops internationally accepted Test Guidelines (TGs) that describe standard experimental procedures. These guidelines make toxicity testing results comparable between different countries and ensure the data can be used for regulatory approval worldwide.

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Toxicity studies are classified based on duration of exposure:

Acute toxicity studies – involve a single exposure or multiple exposures within 24 hours. They determine the immediate harmful effects and estimate the median lethal dose (LD_{50}) or median lethal concentration (LC_{50}).

Sub-acute toxicity studies – involve repeated daily exposures for a short period, typically 28 days, to detect harmful effects from short-term repeated dosing.

Chronic toxicity studies – involve long-term repeated exposure over months to years, to detect slow-developing harmful effects such as organ damage, functional impairment, or carcinogenicity.

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1. Acute Toxicity

Acute toxicity means harmful effects that appear soon after a single dose or multiple doses given within 24 hours.

It shows the immediate danger of a substance and helps find how much is dangerous in one short exposure.

2. Purpose

Find the median lethal dose (LD_{50}) – the amount that kills 50% of test animals.
Identify target organs that get affected quickly.
Understand visible signs of poisoning (symptoms).
Help decide safe starting doses for further studies in animals and humans.

3. OECD Test Guidelines for Acute Toxicity-

A. Oral Acute Toxicity

OECD TG 420 – Fixed Dose Procedure.

OECD TG 423 – Acute Toxic Class Method.

OECD TG 425 – Up-and-Down Procedure.

Procedure:

Give the substance by mouth in a single dose.

Observe animals for 14 days.

Record symptoms, deaths, body weight changes.

At the end, examine organs for damage (necropsy).

B. Dermal Acute Toxicity-

OECD TG 402 – Single skin application (usually on shaved skin of animals) for 24 hours.

Observe for 14 days for redness, swelling, and general illness.

Species: Rat, rabbit.

Application: Test substance applied on shaved skin for 24 hrs (occluded patch).

Observation period: 14 days.

Endpoints: Skin reactions, systemic toxicity, mortality, necropsy.

C. Inhalation Acute Toxicity-

OECD TG 403 – Animals breathe in a fixed concentration of the substance for 4 hours.

OECD TG 436 – Acute toxic class method for inhalation.

Observe for 14 days for breathing problems, coughing, or death.

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Species: Rat.

Exposure: Whole-body/nose-only chambers (single 4-hr exposure).

Observation period: 14 days.

Endpoints: Respiratory distress, clinical signs, body weight, mortality, lung pathology.

4. Common Observations in All Acute Tests

Clinical signs: activity changes, breathing difficulty, tremors, convulsions, skin or eye irritation.

Body weight changes.
Mortality pattern (when and how animals die).
Necropsy findings: swelling, bleeding, organ damage.

5. Importance of Acute Studies-

Provide first safety information about a substance.
Classify chemicals for hazard labels (e.g., "Toxic if swallowed").
Help regulatory bodies decide if the substance is too dangerous for further testing.
Prevent exposing humans to high-risk doses in clinical trials.

Sub-Acute Toxicity Studies (OECD Guidelines)

Sub-acute studies are repeated dose toxicity studies carried out for 28 days (OECD TG 407).

Usually performed in rats by the oral route (can also be dermal or inhalation if relevant).

Animals are divided into control + at least 3 dose groups (low, intermediate, high).

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a) Oral (OECD TG 407)-

Species: Rat (10/sex/group).

Dosing: Daily by gavage/feed/water for 28 days.

Observations: Clinical signs, food/water intake, body weight, blood/urine tests, necropsy, organ weights, histopathology.

(b) Dermal (OECD TG 410 – 21-day study, extended for 28 days)-

Species: Rat, rabbit.

Application: Test substance on shaved skin (6 hrs/day, 5 days/week, for 28 days).

Observations: Local skin effects (redness, irritation), systemic effects, hematology, necropsy, histopathology.

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Observations include-

Clinical signs (behavior, skin, eyes, respiration).
Body weight, food and water intake (weekly).
Blood tests – hematology and biochemistry.
Urine analysis if required.
Functional tests (motor activity, grip strength).

At the end:
Necropsy is performed.
Organs are weighed (liver, kidney, heart, spleen, brain, etc.).
Histopathology of tissues is done.

Purpose: To find toxic effects, target organs, and the NOAEL (No Observed Adverse Effect Level), which helps in planning longer-term studies.

(c) Inhalation (OECD TG 412 – 28-day inhalation)-

Species: Rat.

Exposure: Daily inhalation (6 hrs/day, 5 days/week, for 4 weeks).

Observations: Clinical signs, body weight, respiratory function, hematology, organ weights, histopathology (lungs & systemic).

3. Chronic Toxicity Studies (≥12 months)-

Long-term daily exposure for 1 year or more (OECD TG 452, 453).
Purpose: To detect cumulative, irreversible, or delayed effects, including carcinogenicity.

(a) Oral (OECD TG 452)

Species: Rats (12–24 months), sometimes dogs.
Dosing: Daily by oral route.
Observations: Clinical signs, body weight, food/water intake, hematology, biochemistry, urine, ophthalmology, necropsy, organ weights, histopathology.

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(b) Dermal (Extended OECD TG 411/410 for 12 months or more)
Species: Rats, rabbits.
Application: Daily/regular application to shaved skin (6 hrs/day, 5 days/week).
Observations: Local skin damage, systemic effects, organ toxicity, necropsy, histopathology.

(c) Inhalation (OECD TG 453 – chronic & carcinogenicity)
Species: Rat (up to 24 months).
Exposure: Inhalation chambers, long-term exposure to gases, vapors, aerosols.
Observations: Respiratory distress, body weight, food intake, hematology, pulmonary function, necropsy, lung and systemic organ histopathology.

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Acute eye irritation, skin sensitization, dermal irritation & dermal toxicity studies.

1. Acute Eye Irritation / Corrosion Study

Purpose:

To assess whether a chemical can cause eye irritation (reversible effects like redness, watering, swelling) or eye corrosion/serious damage (irreversible injury like blindness).

OECD Guideline: TG 405

Test System:

Commonly performed on albino rabbits (due to sensitive eyes).

Now alternatives (in vitro models like BCOP – Bovine Corneal Opacity Test, ICE – Isolated Chicken Eye Test) are also used.

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Methodology:

Application – 0.1 mL of liquid / 100 mg of solid is instilled into the conjunctival sac of one eye. The other eye is untreated (control).

Observation Period – Eyes are checked at 1 hr, 24 hr, 48 hr, and 72 hr after application; if needed up to 21 days.

Parameters scored:

Conjunctivae: Redness, swelling (chemosis), discharge.

Cornea: Opacity (cloudiness).

Iris: Damage, abnormal reactions.

Scoring system – Each effect given numerical score → combined to classify the chemical.

Outcome:

Non-irritant, Irritant, or Causes Serious Eye Damage.

Used for labeling & safety warnings (e.g., "Causes serious eye damage").

2. Skin Sensitization Study-

Purpose:

To determine whether a chemical can induce allergic reactions of the skin (contact dermatitis) after repeated exposure.
Unlike irritation, this involves immune system activation (delayed-type hypersensitivity).

OECD Guidelines:

TG 406 – Guinea Pig Maximization Test (GPMT) or Buehler test.
TG 429 – Local Lymph Node Assay (LLNA) in mice.
TG 442 series – Modern in vitro alternatives (e.g., h-CLAT, KeratinoSens).

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Methods-

1. Guinea Pig Maximization Test (TG 406):

Two phases: Induction (exposure with chemical + adjuvant to stimulate immunity) → Challenge (re-exposure).

If skin shows redness, swelling = positive sensitization.

2. Local Lymph Node Assay (LLNA, TG 429):

Mice ears are exposed to chemical for 3 days.

After 5 days, lymph nodes are tested for lymphocyte proliferation (immune activation).

Safer, faster, requires fewer animals.

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Outcome:

Chemical classified as Sensitizer (can cause allergies) or Non-sensitizer.

Use:

Very important in cosmetics, pharmaceuticals, detergents, industrial chemicals to avoid allergy risk in humans.

3. Dermal Irritation / Corrosion Study-

Purpose:
To determine whether a chemical causes direct local damage to skin (erythema, edema, burns, necrosis).
Different from sensitization (which is immune-based).

OECD Guideline: TG 404

Test System:
Traditionally done in albino rabbits, now also in vitro skin models (e.g., reconstructed human epidermis).

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Methodology -

1. Application – A fixed dose of test chemical applied to shaved skin (usually 6 cm^2) under a patch.

2. Exposure time – 4 hours.

3. Observation – At 1 hr, 24 hr, 48 hr, 72 hr and up to 14 days.

4. Scoring system –

Erythema (redness)
Edema (swelling)
Graded numerically.

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Outcome:

Chemicals classified as:

Non-irritant

Irritant (reversible within 14 days)

Corrosive (irreversible damage within 14 days).

Use:

Important for labeling (e.g., "Causes skin irritation" or "Causes severe burns").

4. Acute Dermal Toxicity Study-

Purpose:

-To determine systemic toxicity (whole-body effects) when a chemical is absorbed through the skin.

-Different from irritation/corrosion (which are local).

OECD Guideline: TG 402

Test System:

Carried out in rats or rabbits.

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Methodology-

1. Application – Test chemical applied to shaved skin (about 10% body surface area).
2. Exposure – 24 hours under a semi-occlusive patch.
3. Dosing – Several dose groups are tested.

4.Observation – For 14 days, animals monitored for:
Mortality
Clinical signs (tremors, convulsions, lethargy, skin lesions)
Body weight changes
Necropsy (organ damage).

5. Result – LD_{50} (Lethal Dose for 50% animals) is calculated.

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Outcome:

Chemical classified into toxicity categories (e.g., Category 1 = highly toxic, Category 5 = low toxicity).

Use:

Determines safe handling doses, PPE requirements, and regulatory classification.

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What is Test Item Characterization?

Definition: It means complete identification and description of the substance (test item) that is going to be used in toxicology studies.

It includes its identity, purity, composition, stability, and other critical properties.

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Importance of Test Item Characterization-

1. *Reliability of Study Data*

Toxicology results are only meaningful if the exact nature of the tested substance is known.

Prevents false results due to impurities or degradation.

2. *Regulatory Compliance*

OECD, GLP (Good Laboratory Practice), FDA, and other authorities require proper test item characterization before accepting toxicity data.

3. *Reproducibility*

Well-characterized test item ensures that the same material can be retested or compared in future studies.

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4. Safety & Risk Assessment

Proper chemical identity, purity, and impurity profile help regulators assess true human/environmental risk.

5. Traceability

Ensures the test item used in toxicology studies is the same as the one used in marketed products.

Parameters for Characterization

1. Identity of Test Item

Chemical name (IUPAC), CAS number, structure, molecular weight.

For mixtures → qualitative and quantitative composition.

2. Purity & Impurities

Main active ingredient concentration.

Known impurities, residual solvents, stabilizers.

3. Batch / Lot Information

Batch number, manufacturing date, source, and supplier.

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4. Physical-Chemical Properties

Appearance, color, odor.

Melting/boiling point, density, solubility, partition coefficient (log P), vapor pressure, pH.

5. Stability

Shelf-life under storage conditions.

Stability in vehicles (like water, feed, oil) used in toxicology studies.

6. Concentration Verification

Analytical checks to confirm dosing solutions/suspensions contain the intended concentration.

Methods Used for Test Item Characterization-

1. Chemical Characterization

Spectroscopy Methods:

UV-Vis, IR (infrared), NMR (nuclear magnetic resonance), Mass spectrometry.

Chromatography:

HPLC, GC, LC-MS for purity & impurity profiling.

Elemental Analysis:

For metals/minerals, ICP-MS (Inductively Coupled Plasma-MS).

2. Physical Characterization

Melting point / boiling point.

Density, refractive index.

Particle size distribution (important for nanomaterials, powders).

Hygroscopicity, volatility.

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3. Stability Testing

Real-time & accelerated stability studies.

Stability in feed, water, dosing formulations.

4. Biological Characterization (if needed)

For biologics (proteins, vaccines): amino acid sequencing, electrophoresis, bioassays.

Regulatory Expectations-

OECD & GLP: Full test item characterization must be done before dosing starts.

FDA / EMA: Require Certificate of Analysis (CoA) with all critical parameters.

If characterization is incomplete → study data may be rejected