

### Unit 2: Acute, Repeated Dose and Local Toxicity Studies; Test Item Characterization

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#### 2.1 The logic of general toxicity testing

General toxicity studies answer three questions in sequence: what happens after one exposure (acute), what happens when exposure repeats (sub-acute, sub-chronic and chronic), and which organs pay the price. All of them share one architecture: a control group and at least three dose groups, both sexes, daily observation, scheduled clinical pathology, and a full post mortem with histopathology at the end. The output is a target organ list and a NOAEL for that duration and route, and each longer study builds on the shorter one: the acute study sets the first doses, the 28 day study sets the 90 day doses, and so on towards the clinic.

#### 2.2 Acute toxicity: one dose, fourteen days

An **acute toxicity study** observes the effects of a single dose (or several doses within 24 hours), followed by an observation period of at least 14 days. It records the pattern of signs, their onset, duration and reversibility, deaths and their timing, and gross pathology in animals that die and in survivors at termination. Its modern purpose is classification and first information, not a precise LD50: the classical LD50 test (the deleted OECD TG 401) used many animals to fix one number, and animal welfare pressure replaced it with three alternative methods that classify a substance using very few animals.

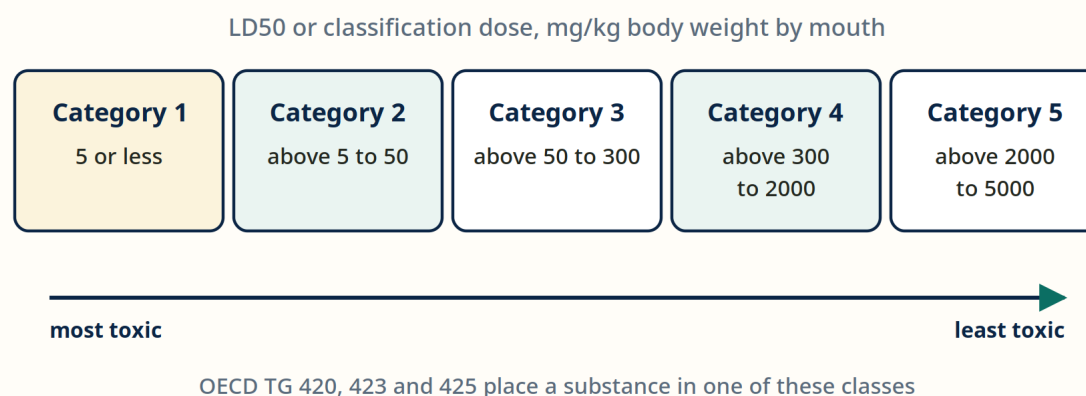
#### 2.3 Acute oral toxicity as per OECD: 420, 423 and 425

Three OECD methods now cover acute oral toxicity, and the differences between them are a favourite examination topic.

- **TG 420, the Fixed Dose Procedure**, uses fixed dose levels of 5, 50, 300 and 2000 mg/kg. A sighting study in single animals finds the dose range that produces clear signs of toxicity without death, and the main study then classifies the substance by evident toxicity at one of the fixed doses. Death is not the intended endpoint.
- **TG 423, the Acute Toxic Class Method**, is a stepwise procedure using the same fixed doses. Three animals (normally females) are dosed at one step; the outcome (death or survival) decides whether the next step goes up, goes down, or repeats, until the substance can be placed in a hazard class.
- **TG 425, the Up and Down Procedure**, doses single animals one at a time, each dose adjusted up or down by a constant factor (the default progression factor is a half log unit, about 3.2) depending on whether the previous animal survived. It estimates an LD50 with a confidence interval using few animals, and is chosen when an LD50 value itself is needed.

All three read across to the **GHS** (Globally Harmonized System) classification of acute oral toxicity: category 1 at 5 mg/kg or below, category 2 above 5 to 50, category 3 above 50 to 300, category 4 above 300 to 2000, and category 5 above 2000 to 5000 mg/kg. The limit test concept runs through all of them: if no toxicity appears at 2000 mg/kg, higher dosing is normally unnecessary.

## GHS acute oral toxicity categories



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Figure: The GHS categories for acute oral toxicity: the fixed doses of the OECD methods, 5, 50, 300 and 2000 mg/kg, are the boundaries between the classes.

### 2.4 Acute dermal and acute inhalational toxicity

- **Acute dermal toxicity (TG 402)** applies a single dose to clipped skin, normally of the rat or rabbit, under a semi occlusive dressing for 24 hours, with 14 days of observation. It matters for substances with occupational or topical exposure and feeds the same GHS classification by the dermal route.
- **Acute inhalation toxicity (TG 403)** exposes rodents, preferably rats, to the test atmosphere for 4 hours in whole body or nose only chambers, at measured concentrations, and classifies gases, vapours and aerosols separately. Atmosphere generation and concentration monitoring are the technical heart of the study: an inhalation result is only as good as the proof of what the animals actually breathed.

### 2.5 Repeated dose studies: the 28 day and 90 day designs

**Sub-acute** in the syllabus language means the repeated dose studies of weeks: the 28 day study is the workhorse (TG 407 for the oral route in rodents, TG 410 for 28 day dermal, TG 412 for 28 day inhalation). Its standard shape is a control group and at least three dose levels, at least 5 males and 5 females per group in rodents, dosed daily for 28 days, with satellite recovery groups at the top dose and control kept for at least 14 further days to show whether effects reverse, persist or appear late.

**Sub-chronic** means the 90 day studies (TG 408 oral in rodents, TG 409 in the non-rodent, usually the dog, TG 411 dermal, TG 413 inhalation). Groups grow to at least 10 males and 10 females per group in rodents, and the clinical pathology and histopathology lists are fuller. The 90 day NOAEL, together with the 28 day result, is the usual basis on which early clinical trials of limited duration are supported, following the ICH M3(R2) rule that the animal studies should equal or exceed the planned human exposure duration.

### Anatomy of a repeated dose toxicity study (28 day or 90 day)

|                     |                                                  |
|---------------------|--------------------------------------------------|
| Control group       | vehicle only, the reference for every comparison |
| Low dose group      | near the expected no effect level                |
| Mid dose group      | geometric step up, bridges the curve             |
| High dose group     | shows clear toxicity, but not death as the rule  |
| Recovery satellites | top dose and control, kept after dosing ends     |

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Daily: clinical signs, mortality, body weight, food consumption

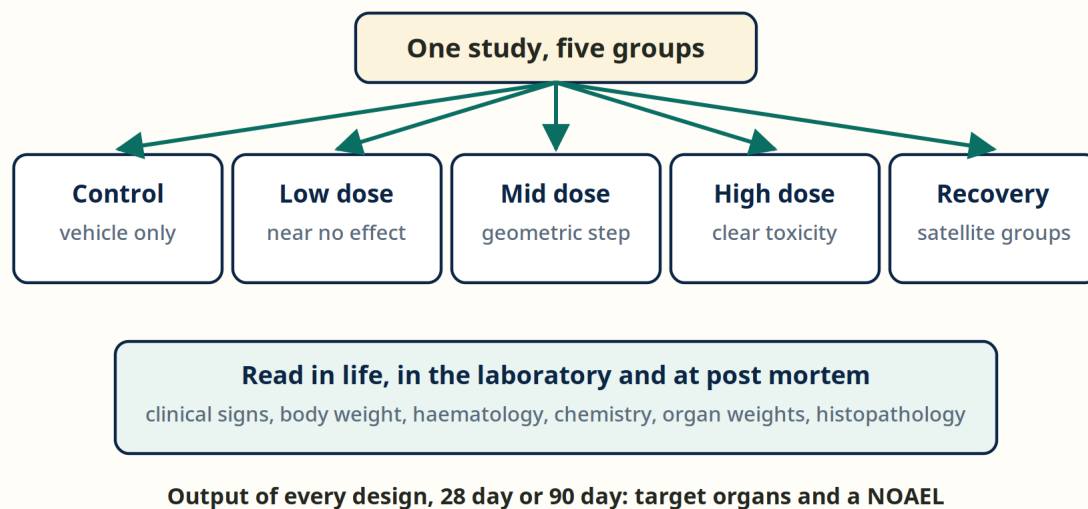
Scheduled: haematology, clinical chemistry, urinalysis

End: gross pathology, organ weights, full histopathology

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Output: target organs + NOAEL for this duration and route

## Anatomy of a repeated dose study



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Figure: The five groups of a repeated dose study and the three layers of observation, in life, in the laboratory and at post mortem, that produce the target organ list and the NOAEL.

## 2.6 Chronic toxicity studies

**Chronic toxicity studies** extend dosing to a major part of the lifespan: TG 452 covers chronic toxicity, normally 12 months in rodents, and TG 453 combines chronic toxicity with carcinogenicity in one design to save animals. Group sizes rise (at least 20 males and 20 females per group for the chronic phase), interim sacrifices may be scheduled, and the questions change with the duration: cumulative injury, slowly developing organ damage, and effects that only months of exposure reveal. These studies support long term human treatment and are commonly run in parallel with the later clinical phases rather than before the first trial.

Dermal and inhalational repeated dose studies belong in the same table of designs, because the syllabus names all three routes together: TG 410 (28 day dermal) and TG 411 (90 day dermal) apply the substance daily to clipped skin, and TG 412 (28 day inhalation) and TG 413 (90 day inhalation) expose animals in chambers, typically 6 hours per day, to measured atmospheres. The route always follows the intended human exposure: a medicine is tested by the route patients will meet, and occupational or accidental routes are added when they are realistic.

## 2.7 What every general toxicity study measures

| Endpoint group     | Typical observations                                                                             | What it detects                                                 |
|--------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| In life            | Clinical signs, mortality, body weight, food and water consumption, ophthalmology                | Overall health, growth effects, eye injury                      |
| Clinical pathology | Haematology, coagulation, clinical chemistry, urinalysis                                         | Liver, kidney, marrow and metabolic injury before it is visible |
| Post mortem        | Gross lesions, organ weights                                                                     | Organ enlargement or atrophy, visible damage                    |
| Histopathology     | Microscopy of a standard tissue list, full in control and high dose, target organs in all groups | The cellular nature of the injury and the NOAEL decision        |

Dose selection follows a consistent logic: the high dose should produce toxicity but not death or severe suffering, the low dose should approach or sit at the expected no effect level, and the spacing between doses is usually geometric. A limit dose of 1000 mg/kg per day applies to repeated dose studies when no toxicity is expected, so animals are not dosed meaninglessly.

## 2.8 Dermal irritation and corrosion: TG 404

The **acute dermal irritation and corrosion study (TG 404)** applies a single dose, normally 0.5 g or 0.5 mL, to the clipped skin of the rabbit for up to 4 hours, and scores erythema (redness) and oedema (swelling) at set times after patch removal over up to 14 days. The scoring separates corrosion (irreversible destruction of the skin) from reversible irritation, and the result drives the GHS skin hazard labelling. In vitro reconstructed human epidermis methods now replace this test for corrosion in many settings (Unit 5).

## 2.9 Skin sensitization: TG 406 and TG 429

**Skin sensitization** is allergic contact dermatitis produced by the immune system, and it is tested differently from irritation because the endpoint is memory, not damage. The classical methods of **TG 406** are guinea pig tests: the maximisation test (GPMT), which uses intradermal induction with an adjuvant plus topical induction, and the Buehler test, which uses repeated occluded topical induction; both end with a challenge application, and a sensitiser is read from the challenge reaction in test animals compared with controls. The modern **TG 429, the Local Lymph Node Assay (LLNA)** in mice, measures proliferation in the lymph nodes draining the application site and gives a quantitative stimulation index, using fewer animals with less distress, and is preferred where it applies.

## 2.10 Acute eye irritation: TG 405

The **acute eye irritation and corrosion study (TG 405)**, the descendant of the Draize test, instils a single dose into one eye of the rabbit, the other eye serving as control, and scores corneal opacity, iris involvement, conjunctival redness and swelling over up to 21 days. Sequential testing in single animals, prior in vitro screening, and weight of evidence approaches now limit its use to cases where the answer cannot be obtained otherwise, because it is among the most distressing of the classical tests. Its replacement by in vitro eye methods is a central case study of Unit 5.

## 2.11 Test item characterization: why the study stands or falls on it

A toxicity study is a comparison between groups that differ only in the dose of one defined substance. If that substance is not what the report claims, every result is worthless, which is why GLP requires the **test item to be characterized** before and during the study. Characterization answers four questions. What is it: identity, established by appropriate analytical methods against a reference standard. How pure is it: purity and the impurity profile, because an impurity, not the drug, may be the toxicant. Is it stable: stability under

the storage conditions of the study and in the dosing vehicle for the period of use. And is each dose what it claims: the concentration, homogeneity and stability of dose formulations are verified by analysis at intervals during the study, a step called dose formulation analysis. Batch number, receipt, storage, dispensing and reconciliation of the test item are all documented, so that the material tested can be traced exactly, a chain of custody for the molecule at the centre of the study.

## 2.12 Test item characterization: the methods

| Question              | Typical methods                                                                                      | Failure it prevents                                |
|-----------------------|------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| Identity              | Spectroscopic and chromatographic comparison with a reference standard (IR, NMR, MS, HPLC retention) | Testing the wrong substance                        |
| Purity and impurities | HPLC or GC assay and related substances, residual solvents                                           | Attributing an impurity's toxicity to the drug     |
| Stability             | Stability indicating assay over storage and in vehicle                                               | Dosing a degraded substance at an unknown strength |
| Dose formulation      | Concentration and homogeneity analysis of dosing preparations                                        | Groups receiving doses different from the plan     |

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