



TOXICOLOGY

- Toxicology is the scientific study of adverse effects that occur in living organisms due to chemicals.
- It involves observing and reporting symptoms, mechanisms, detection and treatments of toxic substances, in particular relation to poisoning of humans.



Organisation for Economic Co-operation and Development



OECD (SINCE 1961)

- The mission of OECD is to promote policies that will improve the economic and social well-being of people around the world.
- Work with governments to understand what drives economic, social and environmental change.
- Set international standards on a wide range of things, from agriculture and tax to the safety of the chemicals.



OECD – INDIA

- India is one of the many non-member economies with which the OECD has working relationships in addition to its member countries. The OECD has been cooperating with India since 1995.
- The OECD Council at Ministerial level adopted a resolution on 16 May 2007 to strengthen the co-operation with India, as with Brazil, China, Indonesia and South Africa, through a programme of enhanced engagement.

TOXICITY STUDIES

- Acute toxicity (14 Days)
- Sub-acute (repeated doses) toxicity (28 Days)
- Sub-chronic toxicity (3 Months)
- Chronic toxicity (6 Months to 2 Years)
- Special toxicity (Carcinogenicity)

OECD Test Guidelines

- ♦ Acute Oral Toxicity Test: OECD TGs 420, 423, 425
 - Fixed Dose Procedure (OECD 420)
 - Acute Toxic Class Method (OECD 423)
 - Up and Down Procedure (OECD 425)
- ♦ Subacute oral toxicity, 28 days; OECD 407
- ♦ Subchronic oral toxicity, 90 days; OECD 408
- ♦ Carcinogenicity Studies (OECD 451)
- ♦ **Chronic Toxicity Studies (OECD 452)**
- ♦ Combined Chronic Toxicity/Carcinogenicity Studies (OECD 453)



ACUTE TOXICITY

Acute toxicity studies are conducted to determine the short-term adverse effects of drug when administered in a single dose, or in multiple doses during a period of 24 h in two mammalian species (one non-rodent).



ACUTE TOXICITY SYMPTOMS

- Altered Respiration
- Weight loss
- Muscle spasm
- Salvation
- Convulsion
- Diarrhea
- Loss of righting reflex
- Tremor
- Lacrimation

WHAT IS LD₅₀ ?

- LD50 represents the individual **dose** required to kill 50% of a population of test animals.
- It is an index determination of medicine and poison's virulence.
- Lower the **LD 50** dose, the more toxic the pesticide.

WHAT IS LC₅₀?

- The **concentrations** of the chemical in air that kills 50% of the test animals during the observation period is the LC50 value..
- Other durations of exposure (versus the traditional 4 hours) may apply depending on specific laws.



LD50/LC50



What this means

higher LD_{50}/LC_{50} = less toxic



lower LD_{50}/LC_{50} = more toxic



TOXICITY CATEGORY	ORAL LD₅₀ (mg/kg)	SIGNAL WORD	APPROX. ADULT LETHAL DOSE (ORAL)
I	0-50	DANGER/ POISON	Few drops to 1 teaspoon
II	50-500	WARNING	1 teaspoon to 1 ounce
III	500-5,000	CAUTION	1 ounce to 1 pint or pound
IV	>5,000	CAUTION	More than 1 pint or pound

Putting it all together.....

High Acute Toxicity

= Category I


= DANGER/POISON

= low LD₅₀/LC₅₀

= small amount of
pesticide can kill or
harm you

METHODS TO DETERMINE LD₅₀

- Karber's method
- Miller and Tainter method
- Lorke's method
- Fixed dose method
- UP and down method

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- A humane endpoint can be defined as the earliest indicator in an animal experiment of severe pain, severe distress, suffering, or impending death.
 - OECD Test Guidelines do not require death as an endpoint.
 - Animals humanely killed during the test will be regarded as dosage-dependent deaths.
 - Three alternative test methods (Guidelines 420, 423, and 425) to the traditional acute oral toxicity test have been adopted by the OECD. One of these, the Fixed Dose Procedure (Guideline 420).

GUIDELINES	NUMBER OF ANIMAL PER TEST	NUMBER OF DEATH PER TEST	AIM
401	Up to 25	Up to 12	-
420	5-7	1	Endpoint-evident toxicity
423	Average 7	2-3	-
425	6-9	2-3	Stopping criteria to limit number of animals used



ALTERNATIVE APPROACHES

- In vitro (test tube) test methods and models based on human cell and tissue culture.
- Computerized patient-drug databases and virtual drug trials.
- Computer models and simulations.
- Stem cell and genetic testing methods.
- Non-invasive imaging techniques such as MRI and CT Scans.
- Microdosing (in which humans are given very low quantities of a drug to test the effects on the body on the cellular level, without affecting the whole body system).

Number	Title	Original adoption	Number of updates	Most recently updated
401	Acute oral toxicity-conventional acute toxicity test	12 may 1981	1	Date of deletion: 20 December 2002
420	Acute oral toxicity- fixed dose procedure	17 July 1992	1	17 December 2001
423	Acute oral toxicity-acute toxic class method	22 march 1996	1	17 December 2001
425	Acute oral toxicity- up and down procedure	21 September 1998	2	23 March 2006



DESIGN OF ACUTE TOXICITY

- 14 days study.
- Study on at least two species.
- One rodent -mice/rat. One non rodent-usually rabbit.
- Dose administered orally & parenterally. Various dose levels to groups of both sexes.
- Dose selection such that causing than less than 50% but not 0% and more than 50% but not 100% mortality.



ADVANTAGES

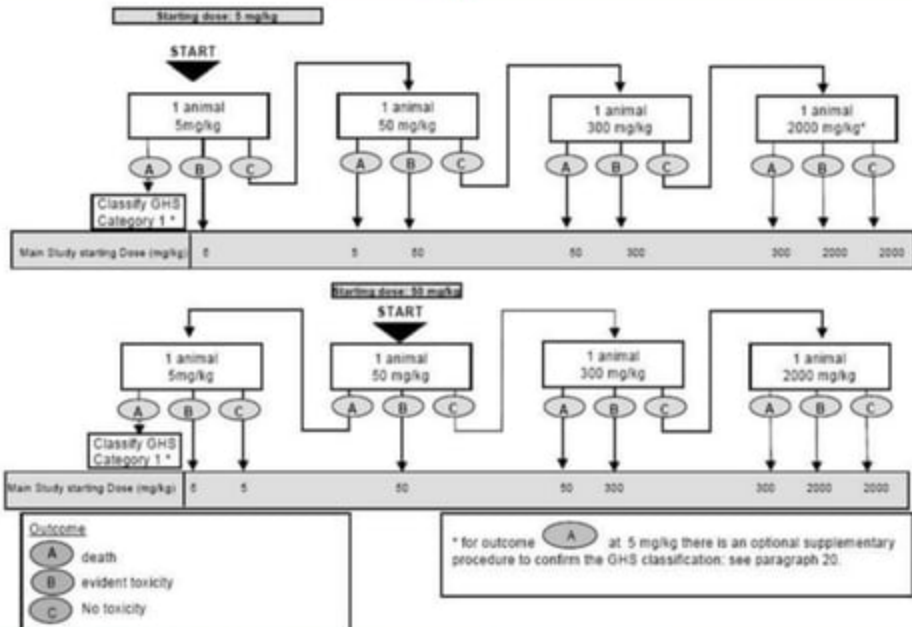
- Reproducible procedure.
- Causes less suffering to the animals.
- Uses only moderately toxic doses, doses expected to be lethal should be avoided.
- Uses fewer animals



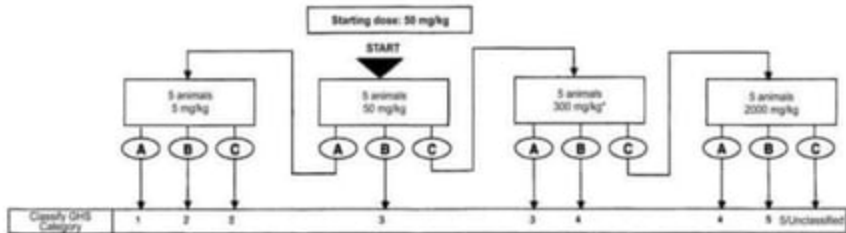
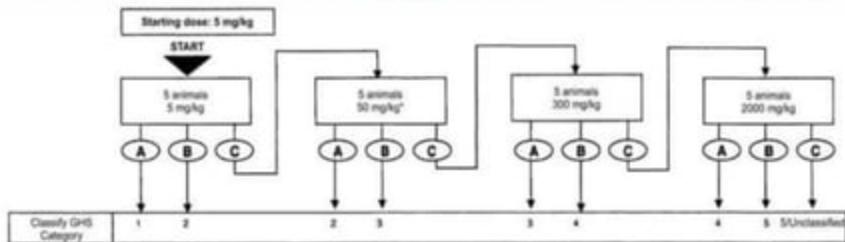
TWO STEP PROCEDURE

1. *Sighting Study*
2. *Main Study*

Sighting Study



Main Study



Outcome:

- A** ≥ 2 deaths
- B** ≥ 1 with evident toxicity and / or 1 death
- C** No evident toxicity and no death

Group size

The 5 animals in each main study group will include any animal tested at that dose level in the sighting study

*Animal welfare override

If this dose level caused death in the sighting study, then no further animals will be tested. Go directly to outcome

A



LIMITATIONS

- Indicated that all are likely to perform poorly for chemicals with shallow dose-response slopes Because Guideline 420 uses evident toxicity as an endpoint instead of death.
- Unusually test substances may cause delayed deaths (5 days or more after test substance administration) mostly in case of 425




CHRONIC TOXICITY

Chronic toxicity is the toxicity produced by a chemical or pharmaceutical when it is exposed in long term (6-12 months)



The main objective are:

- The identification of the chronic toxicity of a chemical
- The identification of target organs
- Characterization of the dose-response relationship
- Identification of a no-observed-adverse-effect level (NOAEL).
- The prediction of chronic toxicity effects at human exposure levels
- Provision of data to test hypotheses regarding mode of action

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- The original Guideline 452 was adopted in 1981.
 - After revision it was adopted in 7 September 2009.
 - The updating of TG 452 has been carried out in parallel with revisions of the Test Guidelines 451 (Carcinogenicity Studies) and 453 (Combined Chronic Toxicity/Carcinogenicity studies) with the objective of obtaining additional information from the animals used in the study and providing further detail on dose selection.
 - This Test Guideline is designed to be used in the testing of a broad range of chemicals, including pesticides and industrial chemicals

Chronic Toxicity Study

Basic Principle of this test



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Pharmacology Concepts
By Rajesh Choudhary

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Description of Methods:

- Animals in each group:
 - Rodents: Rats, mice (M & F-20 animal each sex)
 - Non Rodents-(M & F- 4 animal each sex)
 - The females should be nulliparous and non-pregnant.
- Housing: In India, As per CPCSEA guideline (22°C (+ 3°C), 50-60% RH, 12 h light-dark cycle, feed with standard laboratory diet with water at libitum).
- Preparation of Animals: The animals are randomly selected (weight variation <20%), marked and acclimatize for at least 7 days prior to the start of dosing to avoiding any stress.

- Dose level: Dose levels will generally based on the results of shorter term repeated dose or range finding studies. 3 dose level (2-4 fold interval) will used with an one control (vehicle control) group.
 - Control group- vehicle treated
 - Low dose level
 - Mid dose level
 - Higher dose level \leq evident toxic dose and not more than 1000 mg/kg
- Dose preparation: oral, via gavage (10ml/kg), food or drinking water
- Vehicle: Water/corn oil

Test substance:

- physical nature, purity, and physicochemical properties;
- identification data;
- Source of substance;
- batch number;
- certificate of chemical analysis
- Vehicle (if appropriate): justification for choice of vehicle (if other than water).

Test animals:

- species/strain used and justification for choice made;
- number, age, and sex of animals at start of test;
- Source, housing conditions, diet, etc.
- individual weights of animals at the start of the test.

Test conditions:

- rationale for route of administration and dose selection;
- when applicable, the statistical methods used to analyse the data;
- details of test substance formulation/diet preparation:

Test conditions:

- analytical data on achieved concentration, stability and homogeneity of the preparation
- route of administration and details of the administration of the test substance;
- for inhalation studies, whether nose only or whole body;
- actual doses (mg/kg body weight/day), and conversion factor from diet/drinking water test substance concentration (mg/kg or ppm) to the actual dose, if applicable:
- details of food and water quality.

Results:

- survival data;
- body weight/body weight changes;
- food consumption, and water consumption if applicable;
- toxic response data by sex and dose level, including signs of toxicity;
- nature, incidence (and, if scored, severity), and duration of clinical observations (whether transitory or permanent);
- ophthalmological examination;
- haematological tests;

Results:

- clinical biochemistry tests;
- Urine analysis tests;
- outcome of any investigations of neurotoxicity or immunotoxicity
- terminal body weight;
- organ weights (and their ratios, if applicable);
- necropsy findings:
- detailed description of all treatment-related histopathological findings:
- Absorption data if available;
- Statistical treatment of results, as appropriate

Discussion of results including:

- Dose - response relationships
- Consideration of any mode of action information
- Discussion of any modelling approaches
- BMD, NOAEL or LOAEL determination
- Historical control data
- Relevance for humans

Conclusion



THANK YOU