

# **Chapter 5: ACUTE, SUBACUTE AND CHRONIC TOXICITY IN ANIMALS**

**Year III Pharm.D  
Dr. V. Chitra**

# INTRODUCTION

- A drug is a single substance or mixture of substances used for diagnosis, treatment, mitigation or prevention of disease ; restoring, correcting or modifying the organic functions in man or animals (W.H.O.)
- Agents of these potential activities interfere with biological processes of the host or extraneous etiological agents and hence are toxic substances.
- The object of toxicity testing in the laboratory is to elucidate the toxic properties of drugs

# ACUTE TOXICITY STUDIES

- The Globally Harmonized System (GHS), defines it as "those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours"
- The preferred species for oral and inhalation testing is the rat, and for dermal testing, the rat or rabbit
- Oral administration is the most common form of acute systemic toxicity testing.

Five Organisation for Economic Cooperation and Development (OECD) Test Guidelines (TGs 402, 403, 420, 423, and 425) describe acute systemic testing.

Fixed Dose Procedure (OECD TG 420)

Acute Toxic Class method (OECD TG 423)

Up-and-Down Procedure (OECD TG 425)

Acute Dermal Toxicity OECD TG 402,

Acute inhalation toxicity OECD TG 403.

# INITIAL CONSIDERATIONS

- 🌐 The testing laboratory should consider all available information on the test substance prior to conducting the study.
- 🌐 The identity and chemical structure of the substance
- 🌐 Its physico-chemical properties
- 🌐 The results of any other *in vitro* or *in vivo* toxicity tests on the substance
- 🌐 Toxicological data on structurally related substances;
- 🌐 The anticipated use(s) of the substance

# DESCRIPTION OF THE METHOD

- ■ Selection of animal species
- ■ Housing and feeding conditions
- ■ Preparation of animals
- ■ Preparation of doses

# PROCEDURE

Administration of doses

Limit test at 2000mg/kg

Limit test at 5000mg/kg

Main test

Observations

Body weight

Pathology

# DATA AND REPORTING

- **Data**
- **Calculation of LD<sub>50</sub>**
- **Test report**

The test report must include the following information, as appropriate:

Test substance:

- physical nature, purity, physico-chemical properties (including isomerisation);
- identification data.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the animals,
- number, age and sex of animals
- source, housing conditions, diet etc.



## **Test conditions:**

- details of test substance formulation, including details of the physical form of the material administered;
- details of the administration of the test substance including dosing volumes and time of dosing;
- details of food and water quality (including diet type/source, water source);
- the rationale for the selection of the starting dose.

## **Results:**

- tabulation of response data and dose level for each animal (i.e. animals showing signs of toxicity including mortality, nature, severity and duration of effects);
- tabulation of body weight and body weight changes;
- individual weights of animals at the day of dosing, in weekly intervals thereafter, and at time of death or sacrifice;
- date and time of death if prior to scheduled sacrifice;
- time course of onset of signs of toxicity and whether these were reversible for each animal;
- necropsy findings and histopathological findings for each animal, if available.

## **Discussion and interpretation of results.**

## **Conclusions.**

# SUBACUTE AND CHRONIC TOXICITY TESTING

- 🌐 The Globally Harmonized System (GHS) defines it as "specific target organ/systemic toxicity arising from a repeated exposure"
- 🌐 Repeated dose toxicity testing using oral administration of a test substance in rodents for 28 and 90 days is used to evaluate chronic toxic effects, primarily effects on various organ systems, and to establish a no observed effect level
- 🌐 Chronic toxicity testing consists of oral, dermal, and inhalation subacute repeated dose studies (28-day) and subchronic repeated dose studies (90-day) in rodents.
- 🌐 The endpoints for repeat dose testing consist of an evaluation of clinical observations, blood analysis, whole body gross necropsy, and microscopic examination of all organs and tissues (histopathology)

The objectives of chronic toxicity studies covered by this test guideline include:

The identification of the hazardous properties of a chemical,

The identification of target organs,

Characterisation of the dose:response relationship,

Identification of a no-observed-adverse-effect level (NOAEL) or point of departure for establishment of a Benchmark Dose (BMD),

The prediction of chronic toxicity effects at human exposure levels,

Provision of data to test hypotheses regarding mode of action

- Six OECD Test Guidelines describe short-term repeat-dose toxicity testing:
- Repeated Dose 28-day Oral Toxicity Study in Rodents ([TG407](#))
- Repeated Dose 90-Day Oral Toxicity Study in Rodents ([TG 408](#))
- Repeated Dose Dermal Toxicity: 21/28-day Study ([TG 410](#))
- Subchronic Dermal Toxicity: 90-day Study ([TG 411](#))
- Repeated Dose Inhalation Toxicity: 28-day or 14-day Study ([TG 412](#))
- Subchronic Inhalation Toxicity: 90-day Study ([TG 413](#))

- Initial considerations
- Description of the method,
- selection of animal species
- Housing and feeding conditions
- Preparation of animals
- Preparation of doses
- Observations
- Ophthalmological examination
- Body weight, food/water consumption and food efficiency
- Haematology and clinical biochemistry
- Pathology gross necropsy
- Histopathology

# Test report

The test report should include the following information:

- **Test substance:**

- Physical nature,

- Purity

- Physicochemical properties;

- Identification data;

- Source of substance;

- Batch number

- **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, achieved concentration, stability  
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

Food consumption, calculations of food efficiency and water consumption

Toxic response data by sex and dose level, including signs of toxicity;

Nature, incidence (and, if scored, severity), and duration of clinical observations

Ophthalmological examination;

Haematological tests;

Clinical biochemistry tests;

Urinalysis tests;

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



Statistical treatment of results, where appropriate.

Body weights,

Organ weights,

Feed consumption (or water consumption) and food efficiency.

Draft consultant's proposal. V. 8. OECD TG 452 November, 2008 12

Discussion of results including:

Dose:response relationships

Consideration of any mode of action information

Discussion of any modelling approaches

Historical control data

Relevance for humans

- Conclusions

# REFERENCES

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