Acute, Sub-Acute and Chronic Toxicity As per OECD Guidelines

Acute Toxicity refers 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.

Subacute Toxicity (repeat dose toxicity) focuses on adverse effects occurring after administration of a single dose or multiple doses of a test sample per day given during a period of from 14 to 28 days.

Chronic toxicity defined as adverse effects occurring after the repeated or continuous administration of a test sample for a major part of the life span.

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).

Fig: Animal Toxicity tests

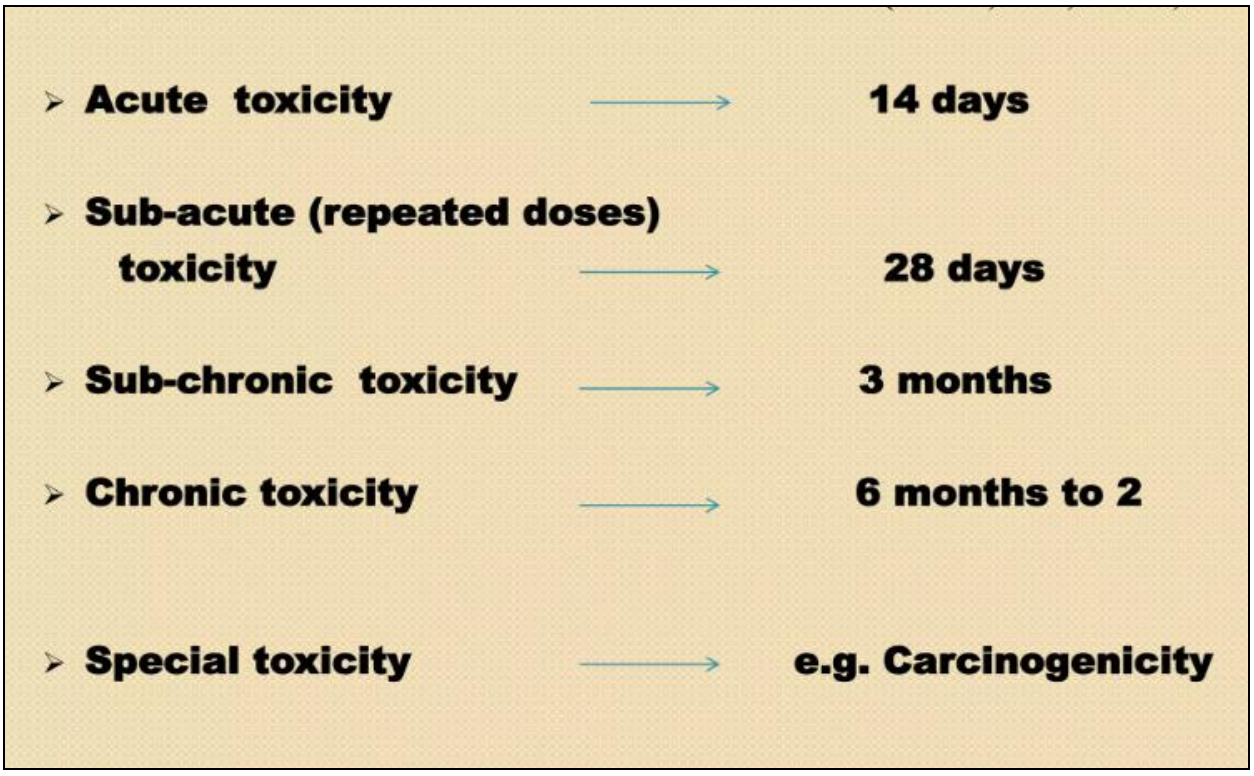


Fig: Acute Oral Toxicity

Table: OECD Guidelines for Acute Oral Toxicity

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 less than 50% but not 0% and more than 50% but not 100% mortality.

ACUTE ORAL TOXICITY OECD GUIDELINES NO.401 (CONVENTIONAL ACUTE TOXICITY METHOD)

- In a study of toxic characteristics of substance, acute oral toxicity testing is initial step.
- Gives information on health hazards.
- Test substance administered orally, in graduated doses to several groups of experimental animals.
- One dose used per group.
- At least 5 rodents at each dose level of same sex are used.
- Observations for effects & death are made.
- After completion of study in one sex, study in another sex is carried out.
- Studies suggested in rodents but can be adopted for studies in non-rodents.

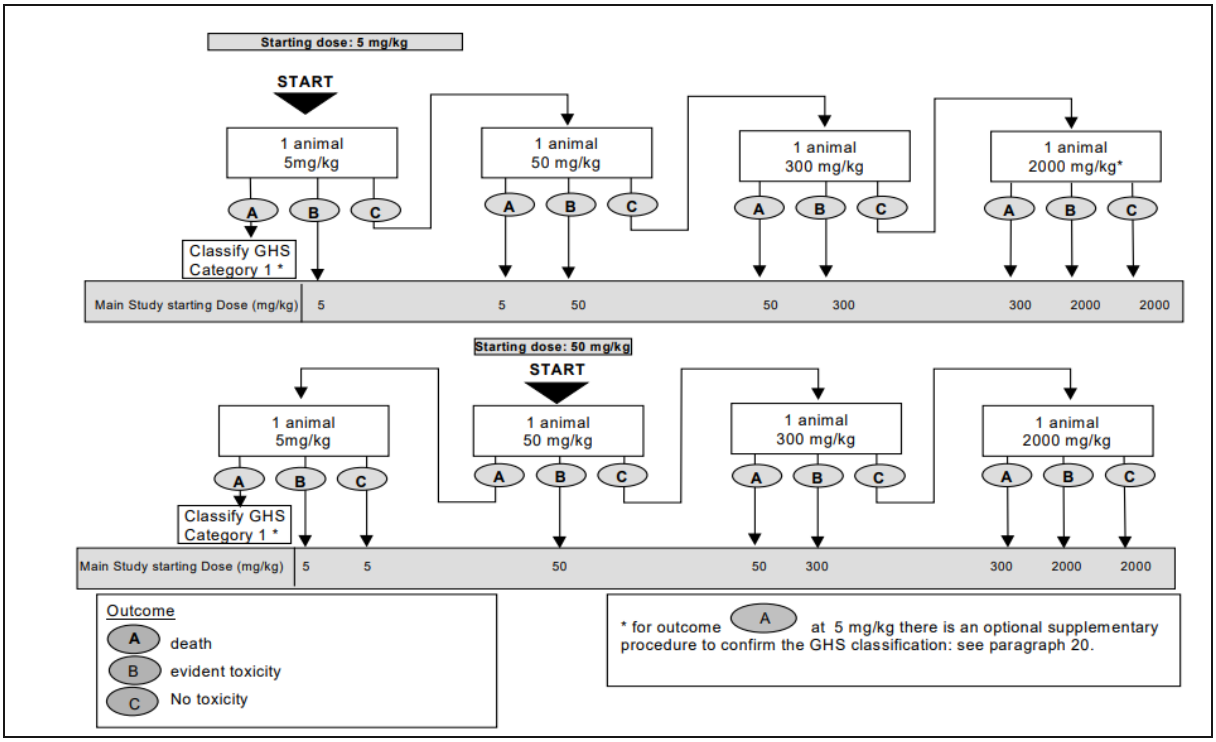
ACUTE ORAL TOXICITY GUIDLINE NO. 420 (FIXED DOSE PROCEDURE)

- New approach in 1984 by British toxicology society based on administration of series of fixed dose levels.
- Instead of death, clear signs of toxicity to animals as end point.
- Adopted as 1st alternative to conventional acute toxicity test.
- Testing in 1 sex usually females is considered sufficient.
- Uses fewer animals.
- Reproducible procedure.
- Causes less suffering to the animals.
- Uses only moderately toxic doses, doses expected to be lethal should be avoided.

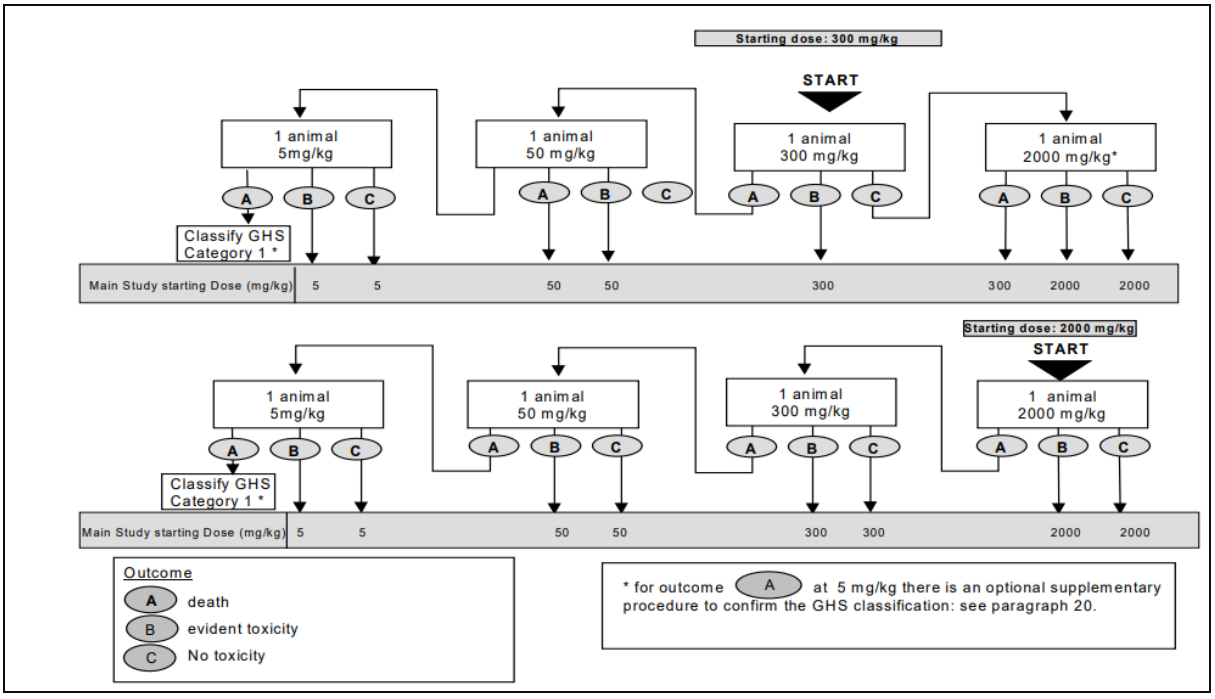
Procedure

Procedure involves 2 step study

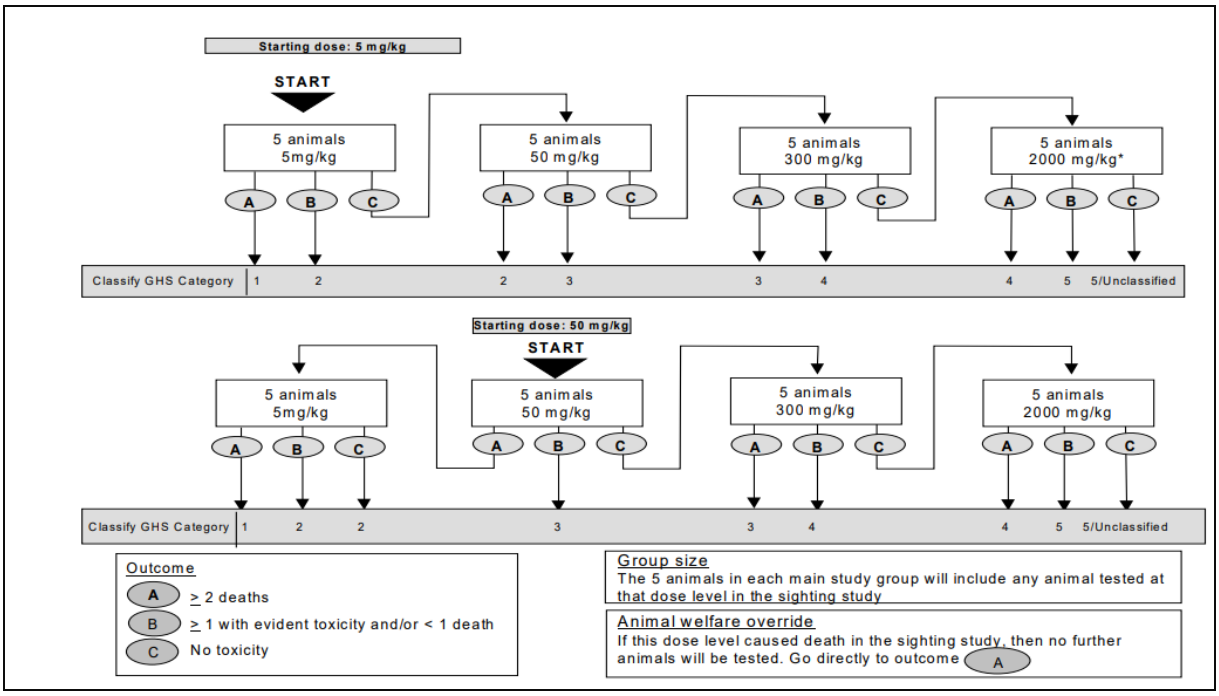
1- sighting study
2- main study



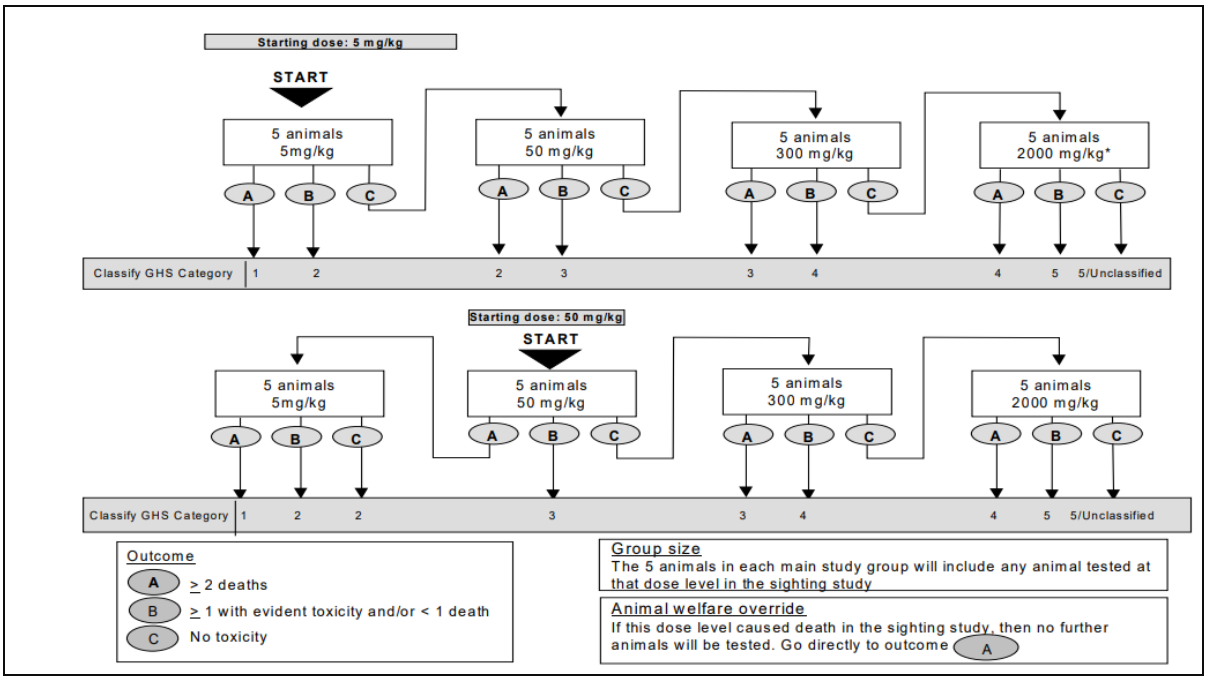
Fig(i): Flowchart for the sighting study.



Fig(ii): Flowchart for the sighting study.



Fig(iii): Flowchart for Main Study.



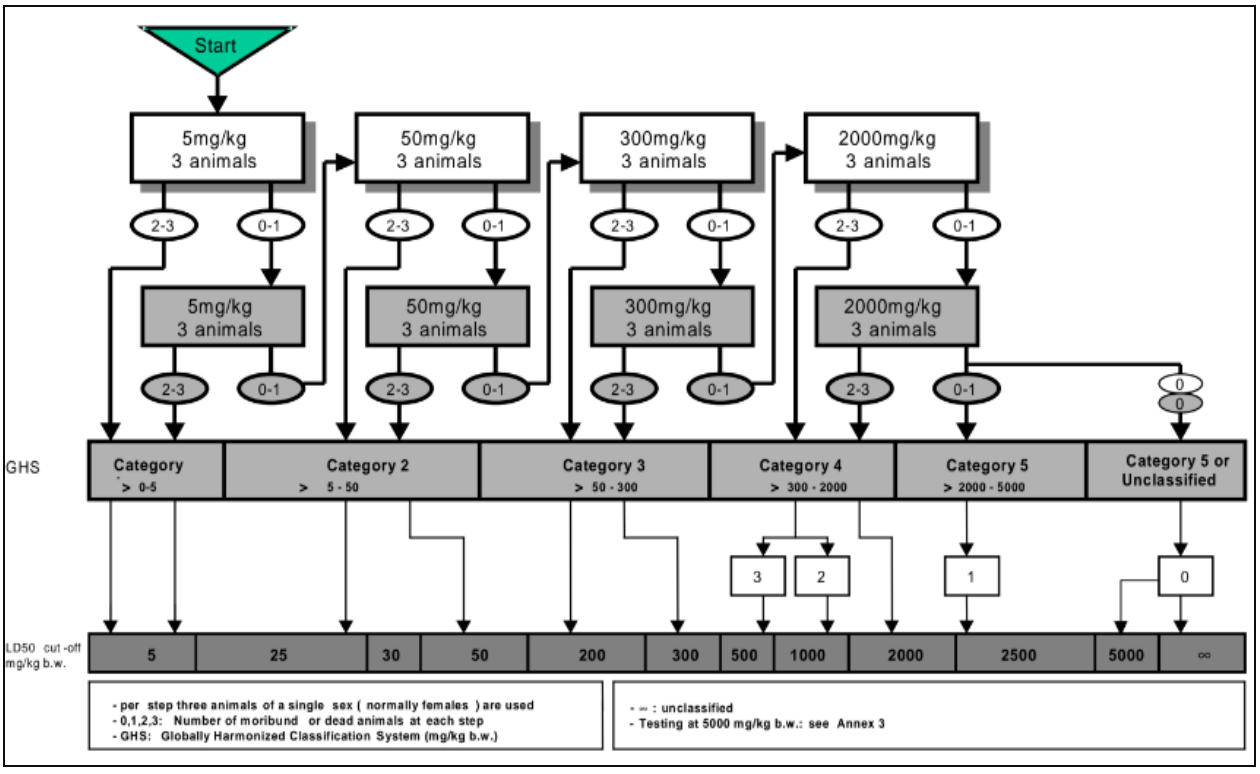
Fig(iv): Flowchart for Main Study.

ACUTE ORAL TOXICITY GUIDLINE NO.423 (ACUTE TOXIC CLASS METHOD)

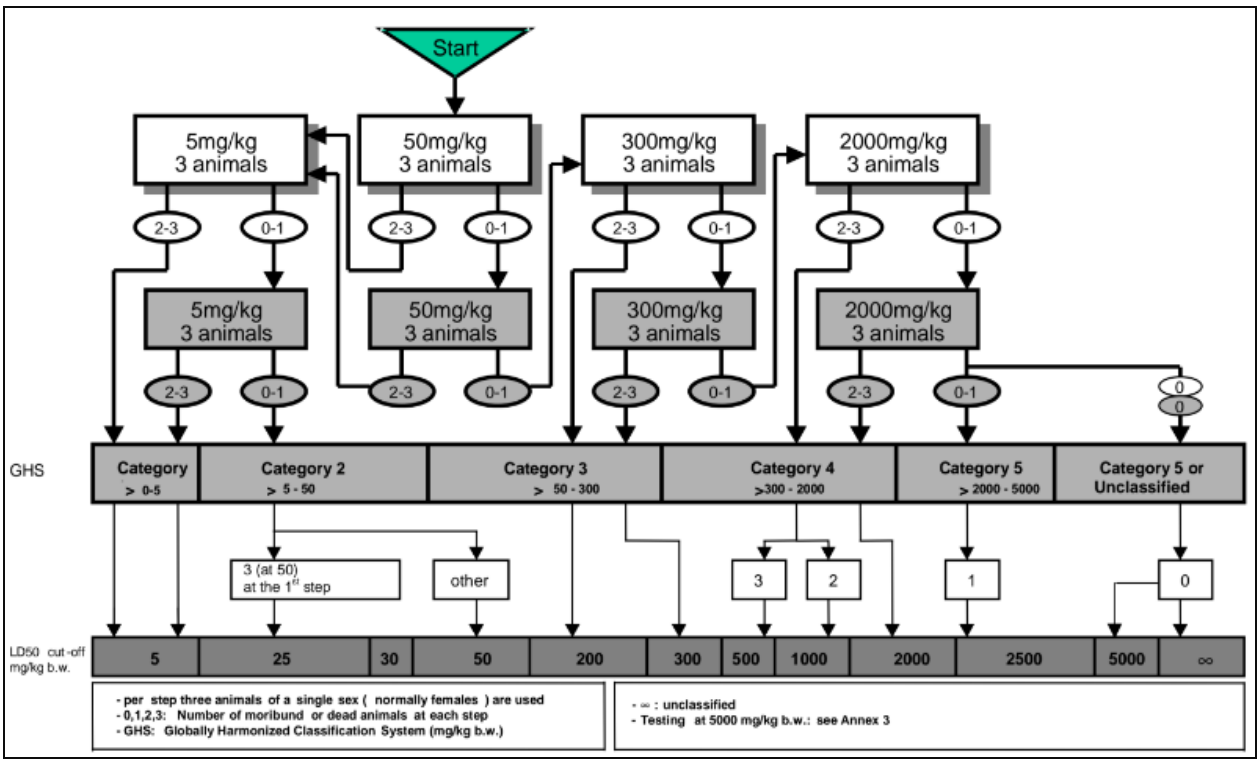
- No sighting studies.
- 3 animals of single sex per step.
- On avg. 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance.
- Not intended to allow the calculation of precise LD50.
- Death of a proportion of animals as the major end point (response).
- Ld50 cut off values are indicated.

PRINCIPLE

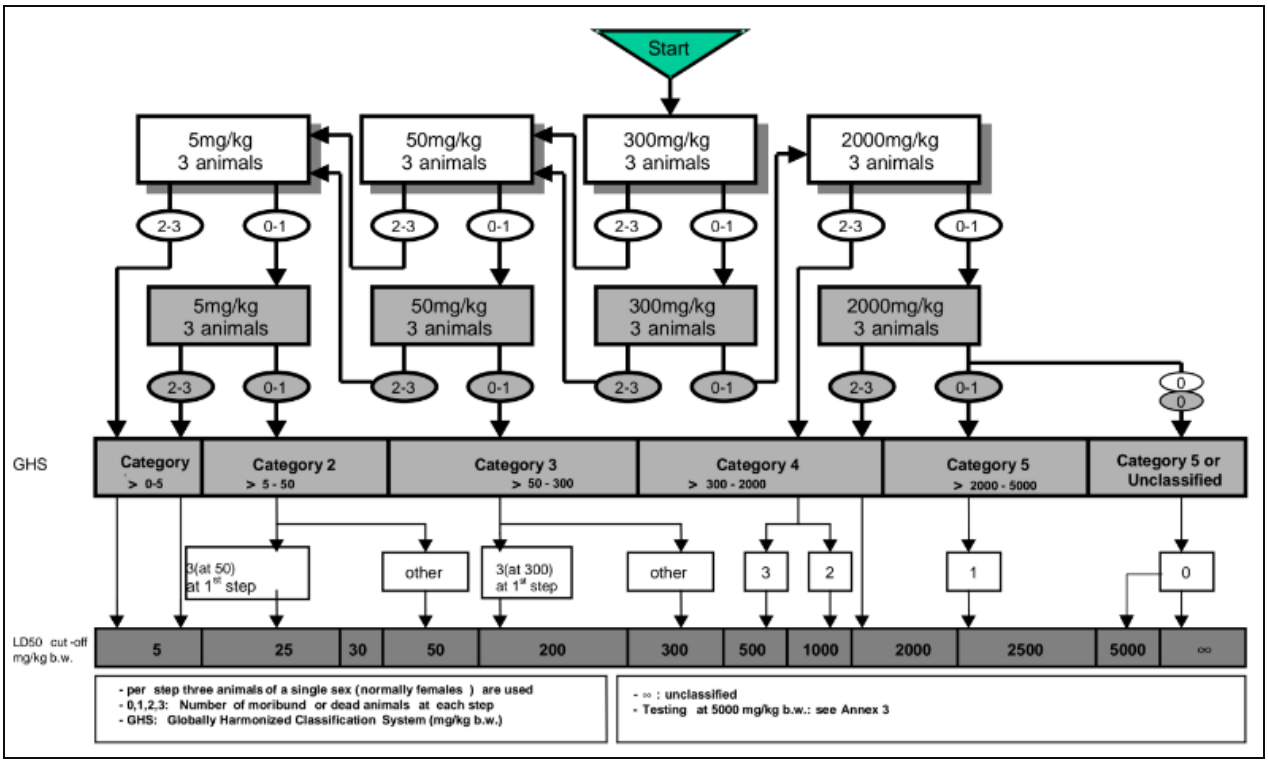
- Stepwise procedure with the use of minimum no. of animals per step.
- Substance administered orally to 2 groups of animals at defined doses.
- 3 animals per step of single sex (normally females).
- Compound related mortality determines the next step.
- Report.



Fig(v): Test procedure with a starting dose of 5mg/kg body weight.



Fig(vi): Test procedure with a starting dose of 50mg/kg body weight.



Fig(vii): Test procedure with a starting dose of 300mg/kg body weight.

ACUTE ORAL TOXICITY GUIDLINE NO.425 (UP AND DOWN METHOD)

- Up and down testing approach was 1st described by Dixon and Mood.
- Bruce in 1985 proposed to use it for acute toxicity determination of chemicals.
- Estimates confidence intervals for LD50.
- In procedure (main test) 1-animal dosed at a time, at minimum of 48 hrs. interval.
- Suggested starting dose is 175 mg/kg or can be selected from 1.75, 5.5, 17.5, 55,175,550,2000mg/kg.
- Animal receives 1st dose a step below the level of the best estimate of LD50.
- Depending upon the outcome for the previous animal, the dose for the next animal is adjusted up or down.
- 5 reversals in 6 consecutive animals when obtained test is terminated.
- No. of animals limited to.

Acute Toxicity Study for Inhalation OECD Guidelines 403

INTRODUCTION

- Acute toxicity study for inhalation was documented as document no. 39 as OECD GUIDELINE FOR THE TESTING OF CHEMICALS
- This revised Test Guideline 403 (TG 403) has been designed to be more flexible, to reduce animal usage, and to fulfill regulatory needs.
- The revised TG 403 features two study types:
  1. A Traditional LC50 protocol and
  2. A Concentration * Time (C x t) protocol.

SELECTION

Selection of the most appropriate species, strain, sex, mode of exposure, appropriate test concentrations include

• the identity,

• chemical structure, and physicochemical properties of the test article;

• results of any in vitro or in vivo toxicity tests;

• anticipated uses and potential for human exposure;

• available (Q)SAR data and toxicological data on structurally related substance.

• The targeted concentrations should not induce severe irritation/corrosive effects, yet reach scientific objective of the test.

PRINCIPLE

- To provide lethality data.
- (e.g., LC50, LC01 and slope) for one or both sexes as needed for quantitative risk assessments. This Guideline offers two test methods.
- Traditional protocol
- groups of animals are exposed to a limit concentration (limit test)
- stepwise procedure • predetermined duration of usually 4 hours.
- (C x t) protocol
- groups of animals are exposed to one (limit concentration)
- multiple concentrations
- multiple durations.

DESCRIPTION OF THE METHOD

1. Selection of animal species

2. Preparation of animals

3. Animal husbandry

4. Inhalation chambers

Selection of animal species: -

• Healthy young adult animals of commonly used laboratory strains should be used.

• The preferred species is the rat and justification should be provided if other species are used.

• both sex.

Preparation of animals

1. Females should be nulliparous and nonpregnant.
2. On the exposure day, animals should be young adults 8 to 12 weeks of age, and body weights should be within ±20% of the mean weight for each sex of any previously exposed animals of the same age.
3. The animals are randomly selected and marked for individual identification. The animals are kept in their cages for at least 5 days prior to the start of the test.

Animal husbandry

- The temperature should be 22±3°C.
- The relative humidity should ideally be maintained in the range of 30 to 70%

- Before and after exposures, animals generally should be caged in groups by sex and concentration,
- When animals are to be exposed nose-only, to be acclimated to the restraining tubes.
- Animals exposed whole-body to an aerosol should be housed individually during exposure
- Lighting should be artificial, the sequence being 12 hours light/12 hours dark.

Inhalation chambers

- The nature of the test article and the objective of the test should be considered when selecting an inhalation chamber.
- The preferred mode of exposure snout-only.
- preferred for studies of liquid or solid aerosols and for vapors that may condense to form aerosols.
- To ensure atmosphere stability when using a whole-body chamber.

EXPOSURE CONDITIONS:

- Administration of concentrations
- Particle-size distribution
- Test article preparation in a vehicle
- Control animals

Administration of Concentration Nose-only exposures may be any duration up to 6 hours in rats. If mice are exposed nose- only, exposures generally should not exceed 4 hours.
Particle-size distribution Particle sizing should be performed for all aerosols and for vapors that may condense to form aerosols. (Metal fumes may be smaller than this standard, and charged particles, fibers, and hygroscopic materials)
Test article preparation in a vehicle vehicle may be used to generate an appropriate concentration and particle size of the test in atmosphere (water). Adequate care should be taken to not contaminate the test material.
Control animals A concurrent negative (air) control group is not necessary.

MONITORING OF EXPOSURE CONDITIONS

1. Chamber airflow
2. Chamber temperature and relative humidity
3. Test article: Nominal concentration
4. Test article: Actual concentration
5. Test article: Particle size distribution

1. Chamber Airflow The flow of air should be carefully Controlled, Continuously monitored, Recorded(at least hourly during each exposure). Oxygen concentration should be at least 19% and carbon dioxide concentration should not exceed 1%. (if not measured)

2. Chamber temperature and relative humidity

• Chamber temperature should be maintained at 22±3°C.
• Relative humidity should be monitored and recorded at least three times for durations of up to 4 hrs, and hourly for shorter durations.

• The relative humidity maintained in the range of 30 to 70%.

3. Test article: Nominal concentration

• mass of generated test

• the total volume of air passed through the chamber system.

• The nominal exposure chamber concentration should be calculated and recorded.

4.Test article:Actual concentration

The actual concentration is the test article concentration at the animals breathing zone in an inhalation chamber. Actual concentrations can be obtained by specific methods (e.g., direct sampling, adsorptive or chemical reactive methods, & analytical characterization) or by nonspecific methods such as gravimetric filter analysisthe test sample should be stored under conditions that maintain its purity, homogeneity, and stability.

5. Test article: Particle size distribution determined at least twice during each 4hour exposure by using a cascade impactor or an aerodynamic particle sizer. A second device, such as a gravimetric filter or an impinger/gas bubbler, should be used in parallel. Particle sizing should be performed for vapors if vapor condensation may result in the formation of an aerosol.

PROCEDURE-

1.Traditional General considerations:

In a Traditional study, groups of animals are exposed to a test article for a fixed period of time (generally 4 hours) in either a nose-only or whole-body exposure chamber. Animals are exposed to either a limit concentration (limit test), or to at least three concentrations in a stepwise procedure (main study). A sighting study may precede a main study unless some information about the test article already exists, such as a previously performed TG 436.

2. Sighting study:

A sighting study is used to estimate test article potency, identify sex differences in susceptibility, and assist in selecting exposure concentration levels for the main study or limit test. When selecting concentration levels for the sighting study, all available information should be used (3 animals/sex may be needed to establish a sex difference). A sighting study may consist of a single concentration, but more concentrations may be tested if necessary.

3. Limit test:

A limit test is used when the test article is known or expected to be virtually non-toxic, Three animals of both sex each. In those situations where there is little or no information about its toxicity, or the test material is expected to be toxic, the main test should be performed. When the GHS Classification System is used, the limit concentrations for gases, vapors, and aerosols are 20000 ppm, 20 mg/L, and 5 mg/L, respectively (or the maximum attainable concentration)

4. Main Test:

A main study is typically performed using five males and five females (or 5 animals of the susceptible sex, if known) per concentration level, with at least three concentration levels. The time interval between exposure groups is determined by the onset, duration, and severity of toxic signs.

C X T PROTOCOL

General considerations

2. Sighting study 3 animal of per sex is used for test and exposure of animal is done for single duration mainly 240 min. When selecting the initial target concentration , the study director should consider the mortality patterns observed in any available TG 436 studies

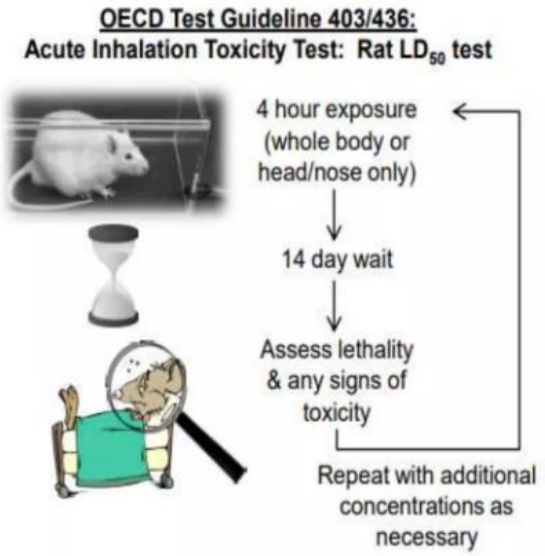
3. Initial Concentration Group of 1 animal/sex is expose to the test article initial conc for time interval of 15, 30, 60, 120 and 240 min Total 10 animals are used in this test. When the GHS Classification System is used, the limit concentrations for gases, vapors, and aerosols are 20000 ppm, 20 mg/L and 5 mg/L, respectively. If less than 50% lethality occurs at the maximum attainable concentration, no further testing is necessary. **Main study**

Figure 1A. Globally Harmonized System (GHS): Acute Toxicity

Category 1	Category 2	Category 3	Category 4	Category 5
				No pictogram
Danger	Danger	Danger	Warning	Warning
330 Fatal if inhaled	**330** Fatal if inhaled	**331** Toxic if inhaled	**332** Harmful if inhaled	**333** May be harmful if inhaled

Figure 2. Globally Harmonized System (GHS): Specific Target Organ Toxicity - Single Exposure

Category 1	Category 2	Category 3
		
Danger	Warning	Warning
370 Causes damage to organs (or state all organs affected, if known)	**371** May Cause damage to organs (or state all organs affected, if known)	**335** May cause respiratory irritation



MAIN STUDY

Exposure Session I –Testing at the limit concentration 1 animal/sex per concentration/time point; 10 animals in total a Target concentration = limit concentration. Expose five groups of animals at this target concentration for durations of 15, 30, 60, 120 and 240 minutes, respectively

Exposure Session II –Main Study 1 animal/sex per concentration/time point; 10 animals in total. Expose five groups of animals at a lower concentration d (1/2L) with slightly lower duration of exposure.

Exposure Session III –Main Study 1 animal/sex per concentration/time point; 10 animals total. Expose five groups of animals at a lower concentration d (1/4L) with slightly longer exposure durations. Exposure Session IV'– Main Study 1 animal/sex per concentration/time point; 10 animals total. Expose five groups of animals at a lower concentration d (1/8L) with slightly longer exposure durations

Exposure Session IV –Main Study 1 animal/sex per concentration/time point; 10 animals total. Expose five groups of animals at a higher concentration e (2L) with slightly shorter exposure durations.

OBSERVATION

- Once daily for 14 days.
- The length of the observation period is not fixed,
- The times at which signs of toxicity appear and disappear are important,
- Individual records being maintained for each animal
- Animals found in a moribund condition should be humanely killed for animal welfare reasons.
- Cage-side observations should include changes in the skin and fur, eyes and mucous membranes,
- Also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior patterns
- Attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Body weights

- Individual animal weights should be recorded once during the acclimatization period, on the day of exposure prior to exposure (day 0), and at least on days 1, 3 and 7 (and weekly thereafter), and at the time of death or euthanasia
- Pathology
- All test animals, including those which die during the test or are euthanized and removed from the study for animal welfare reasons, should be subjected to gross necropsy.

DATA AND REPORTING

Data:

- Individual animal data on body weights and necropsy findings should be provided.
- summarized in tabular form, showing for each test group the number of animals used, the number of animals displaying specific signs of toxicity, the number and time ofanimals found dead during the test or killed for humane reasons, , a description and time course of toxic effect.

TEST REPORT

- The test report should include the following information, as appropriate:
- Description of caging conditions, including: number (or change in number) of animals per cage, bedding material, ambient temperature and relative humidity, photoperiod, and identification of diet
- Species/strain used and justification for using a species other than the rat
- Number, age and sex of animals
- Method of randomization
- Details of food and water quality (including diet type/source, water source)
- Description of any pre-test conditioning including diet, quarantine, and treatment for disease

RESULTS

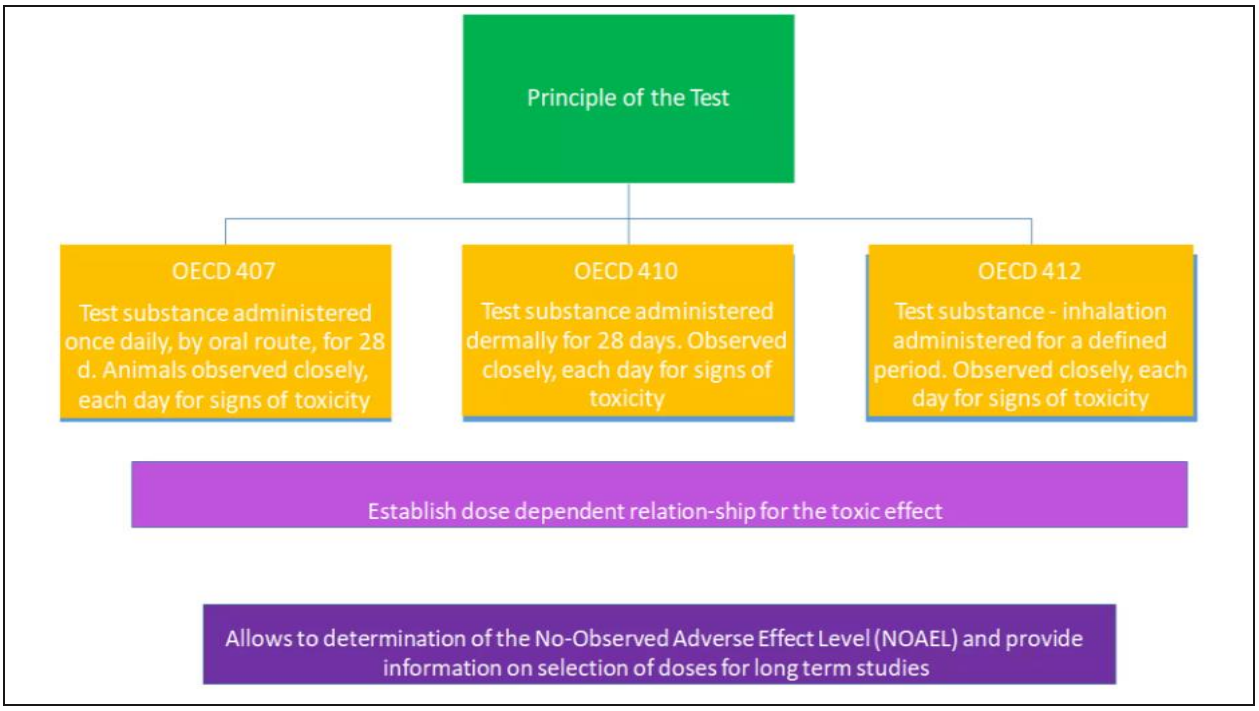
- Tabulation of chamber temperature, humidity, and airflow; - Tabulation of chamber nominal and actual concentration data
- Tabulation of particle size data
- Individual body weights of animals collected on study;
- date and time of death if prior to scheduled euthanasia,
- time course of onset of signs of toxicity and whether these were reversible for each animal • Necropsy findings and histopathological findings for each animal, if available
- Lethality estimates (e.g. LC50, LD01) including 95% confidence limits, and slope (if provided by the evaluation method)
- Statistical relation, including estimate for the exponent n (C x t protocol). The name of the statistical software used should be provided.

SUBACUTE TOXICITY TESTING AS PER OECD GUIDELINES

- OECD Guidelines No. 412: 28-day inhalation Toxicity Study in Rodents
- Toxic-kinetics and systemic toxicity study is also studied.
- Provide robust data for quantitative inhalation risk assessments.
- Subacute inhalation toxicity studies are primarily used to derive regulatory concentrations for assessing worker risk in occupational settings.
- (Q)SAR data and toxicological data on structurally related chemicals.
- The data derived from subacute inhalation toxicity studies can be used for quantitative risk assessments and for the selection of concentrations for chronic studies.

PRINCIPLE

- Accommodate the testing of nanomaterials as well as to reflect the evolving state-of-the-science for the testing of inhaled gases, vapours, and aerosols.
- Broncho-alveolar lavage fluid (BALF) to be performed for all test chemicals
- POE-BALF analysis and lung burden measurements are performed. for all test chemicals(24h) after exposure termination
- When testing a solid aerosol, it is useful to have information on its retention and kinetics in the lung. Dilutions of corrosive or irritating test chemicals may be tested at concentrations that will yield the desired degree of toxicity.



DESCRIPTION OF THE METHOD

1. Selection of Animal Species

Healthy young adult rodents of commonly used laboratory strains should be employed.

The preferred species is the rat. Justification should be provided if other species are used.

2. Preparation of Animals

5 males and females should be exposed at each concentration.

On the day of randomization, animals should be young adults 7 to 9 weeks of age.

Body weights should be within ± 20% of the mean weight for each sex.

allow for acclimatization to laboratory conditions.

3Inhalation Chambers

Subacute inhalation toxicity studies are always performed in dynamic inhalation chambers.

The use of a static inhalation chamber, which has no airflow, is not acceptable.

1*Limit Concentrations*

The maximum concentration tested should consider:

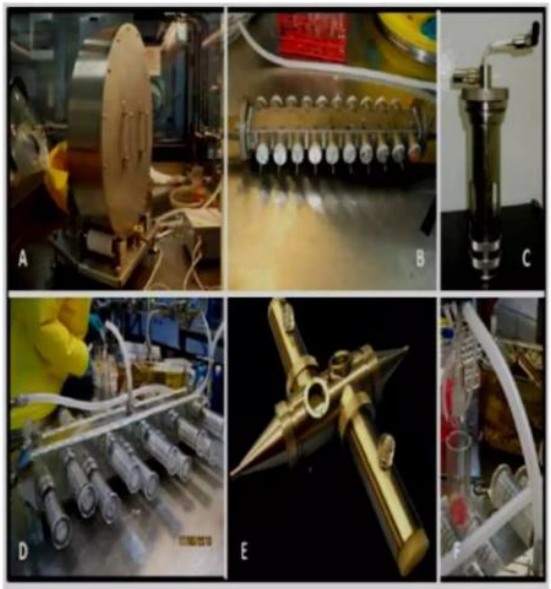
attainable

the need to maintain an adequate oxygen supply, and/or animal welfare considerations.

Up to a maximum concentration of 5 mg/L for aerosols,20 mg/L for vapors, and 20,000 ppm for gases

Dynamic Inhalation Chamber & Static Inhalation Chamber

Dynamic inhalation chamber and laminar hood. (a) Gasoline container, (b) living cage, (c) fans of living cage and (d) laminar hood.



Aerosol apparatus. (A) 40 L Goldberg drum used for aerosol survival studies inside the isolator; (B) A sow used for exposing microthreads to aerosols for aerosol stability studies, in use inside an isolator; (C) A Collison nebuliser used to generate small particle aerosols; (D) Mouse exposure apparatus in use inside an isolator; (E) The marmoset exposure apparatus; (F) An AGI sampling during a mouse exposure inside an isolator.

2 Range-Finding Study

- To inform the selection of concentration levels for a main study.
- No Observed Adverse Effects Concentration (NOAEC), Lowest Observed Adverse Effects Concentration (LOAEC), Maximum Tolerated Concentration (MTC), and/or the benchmark concentration (BMC) in a main study.
- The study director should use a range-finding study to identify the upper concentration that is tolerated without undue stress to the animals.
- A range-finding study should last a minimum of 5 days and generally no more than 14 days, and may include a post-exposure period and animal numbers should be adjusted accordingly.
- When testing poorly soluble particles, it may be necessary for a range-finding study to be longer than 14 days to allow for a robust assessment of test chemical solubility and lung burden.
- The rationale for the selection of concentrations for the main study should be provided in the study report.

Main Study

- The main study consists of at least three test chemical concentration levels and concurrent negative (air) or vehicle controls.
- This guideline differentiates two study designs depending on the nature of the test chemical. Option A, which is generally used for test chemicals (as gas,vapor, aerosol, or a mixture thereof), option B is used when testing chemicals that are likely to be retained in the lungs.

90-DAY (SUBCHRONIC) INHALATION TOXICITY STUDY

INTRODUCTION

- The original sub chronic inhalation Test Guideline 413 (TG 413) was adopted in 1981 (2).
- This revision requires specific measurements of bronchoalveolar lavage fluid (BALF), Measurements of lung burden, should be done when a range-finding study or other relevant information suggests that inhaled test particles are poorly soluble and likely to be retained in the lung.
- A range-finding study (or studies), which primarily is (are) performed to assess concentration levels for the main study should also include BALF analysis, and may also include lung burden measurements
- Sub chronic inhalation toxicity studies are primarily used to derive regulatory concentrations for assessing worker risk in occupational settings.
- This guideline enables the characterization of adverse effects following repeated daily inhalation exposure to a test chemical for 90 days.
- Definitions of technical terms used in this Test Guideline can be found in GD 39 (1).
- Revising this test guideline was to accommodate the testing of nanomaterials as well as to reflect the evolving state-of-the-science for the testing of inhaled gases, vapors, and aerosols.

INITIAL CONSIDERATIONS

- All available information on the test chemical should be considered by the testing laboratory prior to conducting the main study in order to enhance the quality of the study, minimize animal usage, and avoid the need to repeat the study.
- The respirable (or alveolar) fraction of poorly soluble particles that are slowly cleared can accumulate with each consecutive exposure period.
- Dilutions of corrosive or irritating test chemicals may be tested at concentrations that will yield the desired degree of toxicity.
- Animals that are moribund obviously in pain or showing signs of severe and enduring distress should be humanely sacrificed. •DESCRIPTION OF THE METHOD
- Selection of animal species: -
- Preparation of animals • Animal husbandry • Inhalation chambers

TOXICITY STUDIES

- Limit Concentrations
- Range-Finding Study

- A range-finding study should be performed unless sufficient information already exists to perform a robust main study.
- A range-finding study may, for example, provide information regarding analytical methods, particle size distribution, systemic toxicity, toxic- kinetics, test chemical solubility in the lung, BALF data, and estimates of what may be the No Observed Adverse Effects Concentration (NOAEC), Lowest Observed Adverse Effects Concentration (LOAEC), Maximum Tolerated Concentration (MTC), and/or the benchmark concentration (BMC) in a main study.
- A range-finding study may consist of one or more test chemical concentration levels and a control group. Depending on the endpoints chosen, typically no more than 5 males and 5 females should be exposed at each concentration level.
- Should last a minimum of 5 days and generally no more than 28 days,

The main study

- The main study consists of at least three test chemical concentration levels and concurrent negative (air) or vehicle controls
- Each group consists of a minimum of 10 male and 10 female rodents that are exposed to the test chemical for 6 hours per day on a 5 day per week basis for a period of 13 weeks (total study duration of at least 90 days).
- Animals may also be exposed 7 days per week.
- If rodent species other than rats are exposed nose-only,
- A rationale should be provided when using exposure duration less than 6 hours/day, or when it is necessary to conduct a long duration (e.g. 22 hours/day) whole-body exposure study.
- Feed should be withheld during the exposure period unless exposure exceeds 6 hours. Water may be provided throughout a whole-body exposure.

References

1. https://www.slideshare.net/ShitalMagar2/oecd-guidline-on-acute-and-chronic-toxicity
2. https://www.oecd.org/env/test-no-452-chronic-toxicity-studies-9789264071209-en.htm
3. https://unacademy.com/content/nta-ugc/study-material/pharmaceutical-analysis/study-of-acute-sub-acute-and-chronic-toxicity-as-per-oecd-guidelines/
4. https://ifif.org/wp-content/uploads/2019/04/ICCF_GL_02-Subchronic_Toxicity-Step7.pdf