ACUTE, SUB-ACUTE & SUB-CHRONIC INHALATION STUDIES AS PER OECD GUIDELINE

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.

Principle:

TG-403 is designed to obtain sufficient information on the acute toxicity of test article in order to classify it and to provide lethality data from one or both sexes as needed for quantitative risk assessment.

Two guidelines are:-

1) Traditional LC50-

Animals are exposed to limit conc. In a stepwise procedure for a predetermined duration for 4 hrs.

2) Concentration * time (C*T) protocol-

Animals are exposed to one (limit) or a series of conc. Multiple duration.

Description of method:

- Selection of animal species
- Preparation of animals
- Animal husbandry
- Inhalation chambers

1) SELECTION OF ANIMALS-

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.

2) PREPARATION OF ANIMALS-

Females should be nulliparous and nonpregnant, 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.

The animals are kept in their cages for at least 5 days prior to the start of the test.

3) ANIMAL HUSBANDRY-

- The temperature should be 22±3°C.
- The relative humidity should ideally be maintained in the range of 30 to 70%. Animals should generally be caged sex wise and no. of animals in a group should be minimum one.
- When animals are to be exposed nose-only, to be acclimated to the restraining tubes, it may be necessary for them to be acclimated to the restraining cage.
- 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.

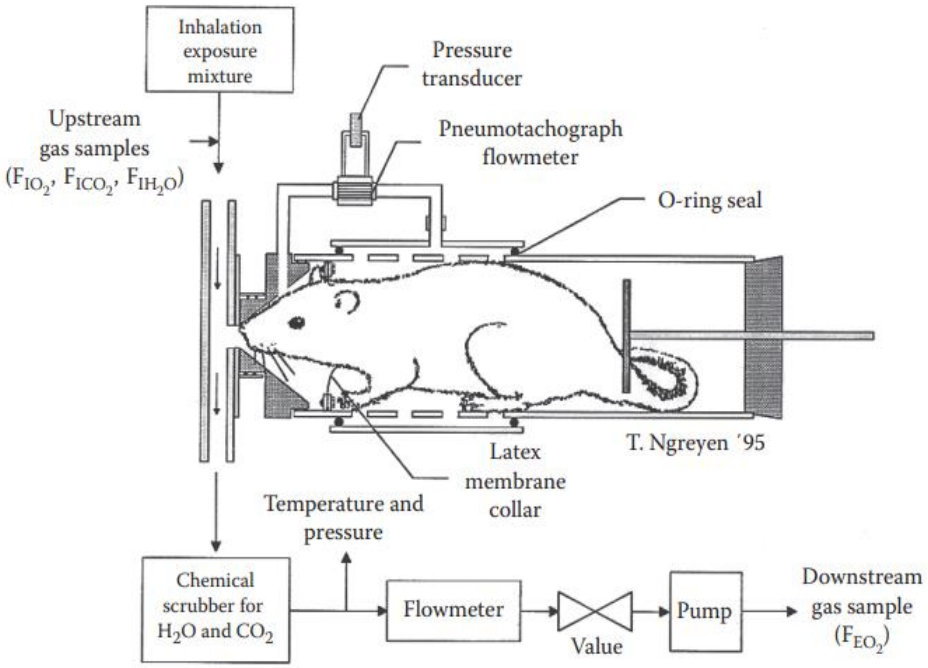
4) INHALATION CHAMBERS-

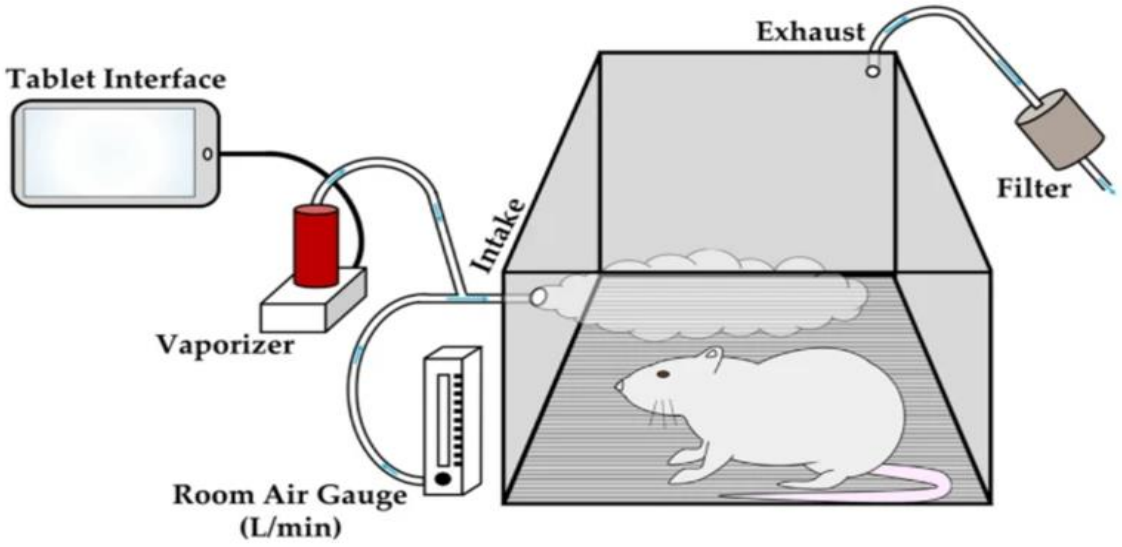
It depends upon nature and objectives of the test articles-

Two types-

1)- NOSE ONLY

2)- WHOLE BODY EXPOSURE





Exposure conditions:

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

1) 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.

2) Particle-size distribution:

Particle sizing should be performed for all aerosols and for vapour that may condense to form aerosols. (Metal fumes may be smaller than this standard, and charged particles, fibers, and hygroscopic materials)

3) 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.

4) Control animals:

A concurrent negative (air) control group is not necessary.

Monitoring of Exposure Condition:

- Chamber airflow
- Chamber temperature and relative humidity
- Test article: Nominal concentration
- Test article: Actual concentration
- Test article: Particle size distribution

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).

CHAMBER TEMP AND RRLATIVE 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%.

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.

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 analysis the test sample should be stored under conditions that maintain its purity, homogeneity, and stability.

TEST ARTICLE: PARTICLE SIZE DISTRIBUTION

It determined at least twice during each 4hour exposure by using a cascade impactor or an aerodynamic particle sizer.

Procedure:

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.

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.

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)

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*T Protocol

General considerations

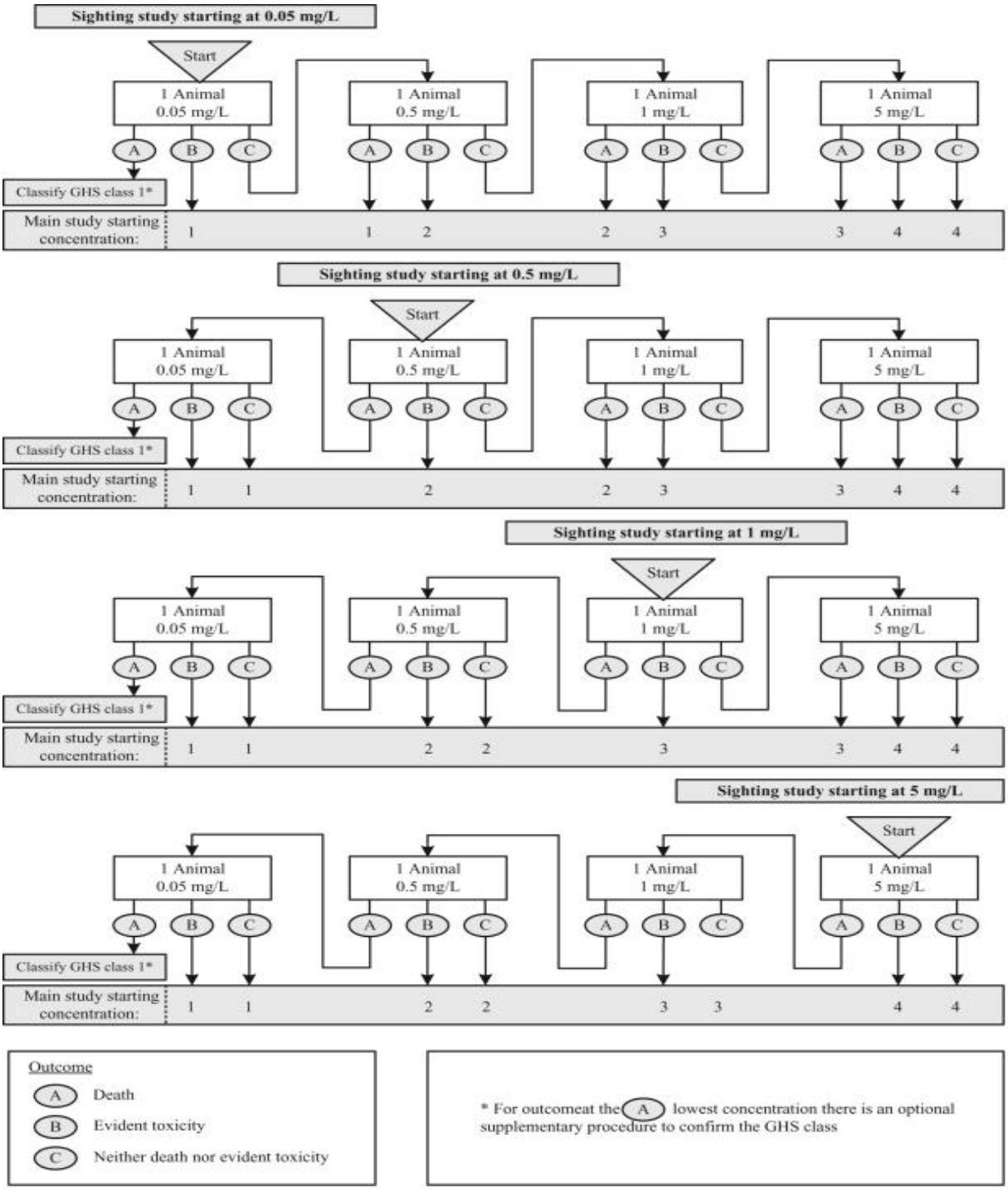
Sighting study

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

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:

- **Exposure Session I** –Testing at the limit concentration 1 animal/sex per concentration/time point. 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 for 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 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 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 & 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 of animals 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.

Result:

- 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.

SUB-ACUTE INHALATION TOXICITY STUDIES

- 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 assessment
- 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:

- **OECD 412-** test substance inhalation administered for a defined period. Observed closely, each day for signs of toxicity.
- Establish dose-dependent relationship for the toxic effect.
- Allows to determination of the NOAEL and provide information on selection of doses for long term studies.
- 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 method:

- Selection of animal species.
- Preparation of animals.
- Inhalation chambers.
- Limit concentration.

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, vapour, aerosol, or a mixture), option B is used when testing chemicals that are likely to be retained in the lungs.

Observation:

BRONCHOALVEOLAR LAVAGE

- BAL should be performed at PEO-1 (within 24 hrs. at termination of exposure) and at one post- exposure interval (PEO-2).
- When testing a poorly soluble aerosol, BAL should be performed at PEO-1 in both sexes and in female animals at PEO-2 if a recovery group is scheduled.
- The right lung is generally preferred for lavage. The duration of the post-exposure period and the timing of the PEOs are determined by the study director based on findings in the range-finding study and other available relevant information.
- The mandatory BALF analysis encompasses the following parameters:
- Lactate dehydrogenase, total protein or albumin cell counts, lymphocytes, neutrophils and eosinophils.

LUNG BURDEN

- When testing poorly soluble solid aerosols, measurement of lung burden can provide clarity on the retained dose.
- Males are used because they have higher minute volume than females and may thus have greater lung burdens.
- To obtained clear information on lung clearance kinetics, the same lung (the right lung is recommended) should be measured for lung burden at all post-exposure time points.

OPTHALMOLOGICAL EXAMINATION

- Refractive media, iris and conjunctivae.

Data & Reporting:

- Individual animal data on body weights, food consumption, clinical pathology, BALF analysis, gross pathology, organ weights, lung burden(when evaluated) and histopathology should be provided for both the range-finding and the main study.
- Clinical observation data should be summarized in tabular form showing for each test group the no. of animals used, the no. of animals displaying specific signs of toxicity, the no. of animals found dead during the test, time of death of individual animals, a description and time course of toxic effects, reversibility and necropsy findings.

Result:

- The respirability of aerosol particles in light of the overall findings should be addressed, especially if the MMAD std. could not be met.
- The consistency of methods used to determine analytical and nominal concentrations, and the relation of these concn should be included in the overall assessment of the study.

SUB-CHRONIC INHALATION STUDIES

- The original sub chronic inhalation Test Guideline 413 (TG 413) was adopted in 1981.
- 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.
- 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 CONSIDERATION

- 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
- Main study
- 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,

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).
- 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.