ACUTE, SUB-ACUTE & SUB-CHRONIC DERMAL TOXICITY STUDIES AS PER OECD GUIDELINE

ACUTE DERMAL TOXICITY STUDIES:

Acute dermal toxicity is the adverse effect caused by a substance following a single uninterrupted exposure by dermal application over a short period of time (24 h or less).

The original acute dermal toxicity guideline TG 402 was adopted in 1987.

A revised TG 402 was considered timely because-

Testing in one sex (usually females) is generally considered sufficient.

The step wise procedure in OECD test guideline 402, with use upto 3 animals of a single sex per step, has been adopted from acute toxic class method and the fixed dose procedure set out in the OECD test guideline 420.

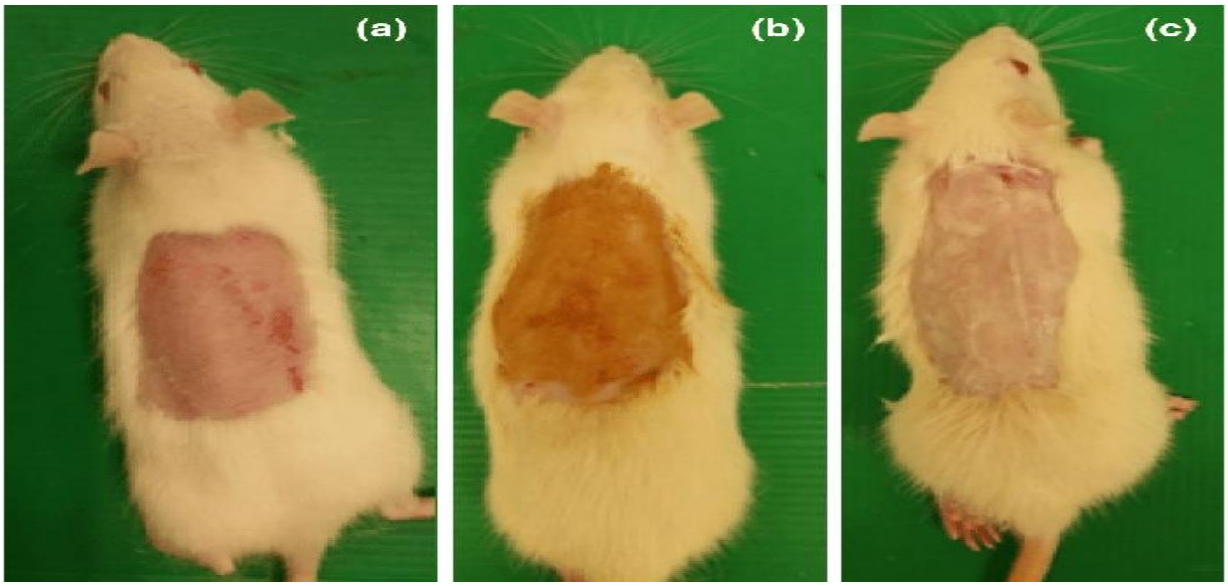
Test chemical is administered to single animal in sequential manner with two animals used at any selected dose level in the main study.

DESCRIPTION OF METHOD:

- **Selection of animal species:** Adult rats (female), Female should be nulliparous and non pregnant.
- **Weight:** (200-300g) & Animals with healthy, intact skin are required.
- **Age:** 8 – 12 weeks.
- **Housing and feeding condition as per CPCSEA.**

- *Temperature*: 22°C (±3 °C).
- *Relative humidity*: 30%-70%.
- 12 hrs light, 12 hrs dark cycle.
- *Feeding*: conventional laboratory diets and unlimited supply of drinking water.

- **Preparation of animals**
  The animals are acclimatized to the laboratory conditions for ate least 5 days prior to the start of the study.
  On the day before administration of the test chemical, all fur should be removed from the dorsal/flank area of the test animals(i.e. at least 10% total body surface) by closely clipping.



PROCEDURE:

ADMINISTRATION OF DOSES

- The test chemical should be applied as uniformly as possible over the exposed area of dorsal/flank skin.
- Test chemical should be held in contact with the skin by a porous gauge dressing and non-irritating tape throughout a 24 hrs exposure period.
- **Solids:** Pulverized before used and moistened with distilled water.
- **Liquids:** Applied directly.
- All animal should normally be observed for at least 14 days.

RANGE FINDING STUDY:

When there is no or insufficient information about test chemical, a dose range study perform by using one animal at starting dose of 200mg/kg body weight is recommended to minimize animal use and optimize the study design.

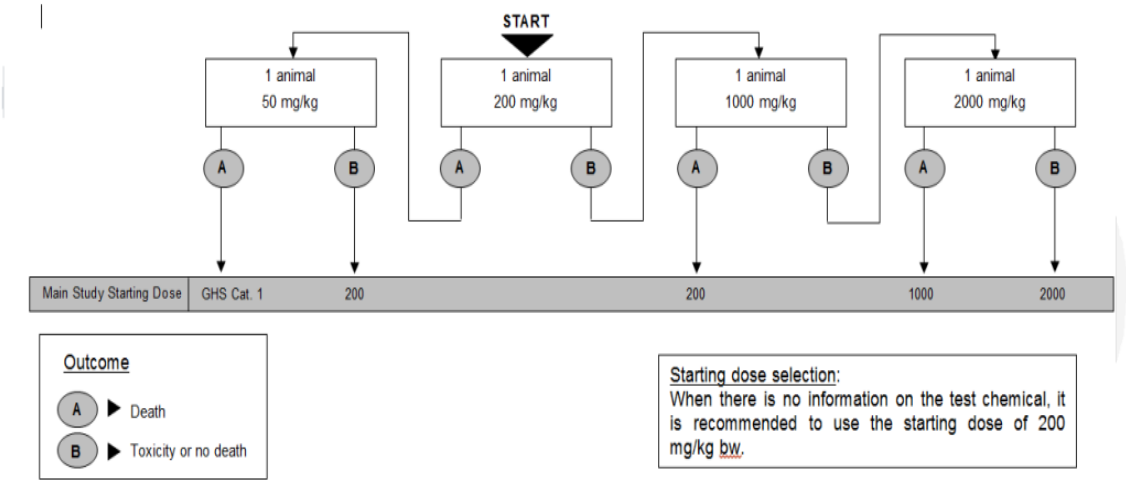
Based on the outcome in range finding study, the main study can be conducted with 2 further animals to confirm the classification outcome.

If information is available for the test chemical, a different starting dose may be chosen i.e. 50, 200, 1000, 2000 mg/kg body wt. following the same procedure based on the GHS category.

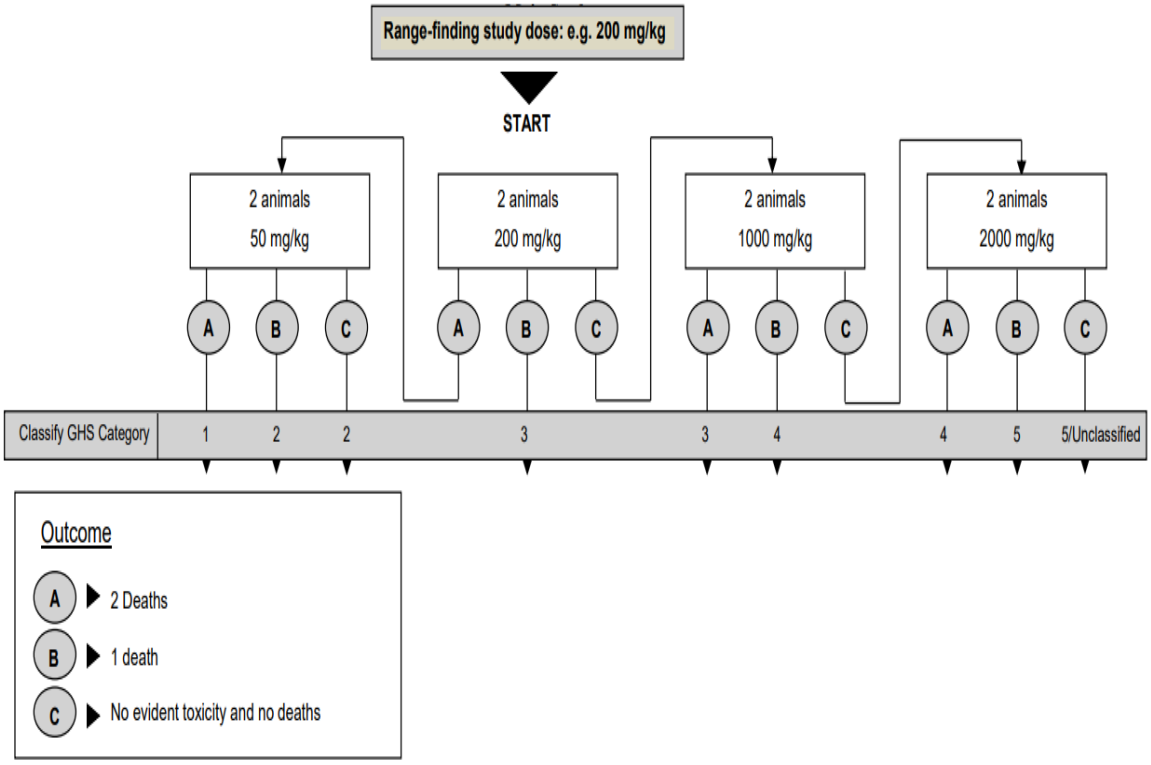
ANNEX 2

FLOWCHART FOR THE TESTING PROCEDURE

Range-Finding Study



MAIN STUDY



GHS- CLASSIFICATION CHART FOR ORAL AND DERMAL TOXICITY-

GHS Category	Classification Criteria			
	Oral		Dermal	
	LD_{50} (mg/kg body weight)	Hazard statement	LD_{50} (mg/kg body weight)	Hazard statement
Category 1	< 5	Fatal if swallowed	< 50	Fatal in contact with skin
Category 2	5 - 50	Fatal if swallowed	50 - 200	Fatal in contact with skin
Category 3	50 - 300	Toxic if swallowed	200 - 1000	Toxic in contact with skin
Category 4	300 - 2000	Harmful if swallowed	1000 - 2000	Harmful in contact with skin
Category 5	2000 - 5000	May be harmful if swallowed	2000 - 5000	May be harmful in contact with skin

Source: (UN 2011)

OBSERVATION:

Animals are observed immediately after dosing at least once during the first 30 minutes, periodically during the first 24hrs, with special attention given during the first 2 to 6 hrs after beginning the exposure period, daily thereafter for total 14 days.

OBSERVATIONS should include:

- Changes in skin and fur
- Eyes and mucus membranes
- Respiratory, circulatory, ANS & CNS
- Somatomotor activity and behavioral pattern
- Attention should be directed to be observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

DATA & REPORTING:

DATA

- Individual animal data should be provided
- Additionally, all data should be summarized in tabular forms.

TEST REPORT: Test report must include-

- Species/strain used.
- Toxic response data by sex and dose.
- Time of death the study or whether animals survived to termination.
- Toxic effects and the time of observation of each abnormal sign and its significance.
- Food and body weight data.
- Clinical biochemistry test.
- Histopathological description.
- Discussion & interpretation of results
- Conclusion

REPEATED DOSE DERMAL TOXICITY STUDIES-

SUB ACUTE DERMAL TOXOCITY STUDIES (410)

- **INTRODUCTION**

- In the assessment and evaluation of toxic characteristics of a chemical the determination of subchronic dermal toxicity may be carried out after initial information on toxicity has been obtained by acute testing.
- There is sufficient similarity b/w the considerations involved in the conduct of a 21 days or 28 days repeated dose dermal study allow one guideline to cover both test durations.

PRINCIPLE:

The test substances is applied daily to the skin in graduated doses to several groups of experiment animals, one dose per grp, for a period of 21/28 days.

During the period of application the animals are observed daily to detect signs of toxicity.

Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied.

DESCRIPTION OF MEHTOD:

Selection of animal species: Adult rats (female), Female should be nulliparous and non pregnant.

Weight: (200-300g) & Animals with healthy, intact skin are required.

Age: 8 – 12 weeks.

Housing and feeding condition as per CPCSEA.

- *Temperature*: 22℃ (±3 ℃).
- *Relative humidity*: 30%-70%.
- 12 hrs light, 12 hrs dark cycle.
- *Feeding*: conventional laboratory diets and unlimited supply of drinking water.

PROCEDURE:

The animals are treated with the test substance, ideally for at least 6 hrs per day on a 7-days per week basis, for a period of 21/28 days.

Application on a 5-days week basis is considered to be acceptable.

Animals in a satellite grp scheduled for follow-up observation should be kept for a further 14 days without treatment to direct recovery form, or persistence of toxic effects.

OBSERVATION:

A careful clinical examination should be made at least once each day. Additional observation should be made daily with appropriate actions taken to minimize loss of animals to the study, e.g. necroscopy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals.

CLINICAL OBSERVATIONS:

- Haematological parameters
- Clinical biochemistry determination
- Gross necropsy
- Histopathology

DATA & REPORTING:

DATA

- Individual animal data should be provided
- Additionally, all data should be summarized in tabular forms.

TEST REPORTING: must be include

- Specimen/ strain used & toxic response data.
- Time of death during the study or animal survive termination.
- Toxic effects and the time of observation of each abnormal sign and its subsequent course and food & body wt. data
- Haematology tests employed and results baseline data
- Clinical biochemistry & necropsy finding.
- Discussion & interpretation of results
- Conclusion.

SUB-CHRONIC DERMAL TOXICITY STUDIES: (412)

- **SUBCHRONIC DERMAL TOXICITY:-** It is the adverse effects occurring as a result of the repeated daily dermal application of a chemical to experimental animal for part (not exceeding 10 %) of a life span.

PRINCIPLE:

The test substance is applied daily to the skin in graduated doses to several groups of experimental animals, one dose per group, for a period of 90 days.

During the period of application the animals are observed daily to detect signs of toxicity.

Animals while die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied.

DESCRIPTION OF MEHTOD:

Selection of animal species: Adult rats (female), Female should be nulliparous and non pregnant.

Weight: (200-300g) & Animals with healthy, intact skin are required.

Age: 8 – 12 weeks.

Housing and feeding condition as per CPCSEA.

- *Temperature*: 22°C (±3 °C).
- *Relative humidity*: 30%-70%.
- 12 hrs light, 12 hrs dark cycle.

- *Feeding*: conventional laboratory diets and unlimited supply of drinking water.

PROCEDURE:

The animals are treated with the test substance, ideally for at least 6hrs per day on a 7-day per week basis, for a period of 90 days.

However, based primarily practiced considerations, application on a 5 day per week basis considered to be acceptable.

Animals in a satellite group scheduled for follow-up observations should be kept for at least a further 28 days without treatment to detect recovery from, or persistence of, toxic effects.

OBSERVATION:

A careful clinical examination should be made at least once each day.

Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, e.g. necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals.

CLINICAL OBSERVATION:

- Opthalmological examination
- Haematology parameters
- Clinical biochemistry determination
- Gross necropsy
- Histopathology

DATA & REPORTING:

DATA:

- Individual animal data should be provided.
- Additionally all data should be summarized in TF.

TEST REPORT: it must be include

- Species & strain
- Time of death during the study of whether animals survived to termination.
- Toxic effects, food and body weight data.
- Haemotological test
- Discussion & interpretation of results
- Conclusion.