TOXICITY AND GENOTOXICITY STUDIES

1.0 Acute Toxicity Studies

1.1 Acute Toxicity and Toxicokinetic Study in Rat and Mice

In order to determine LD_{50} (cut-off) of test item, animals are dosed with one or more doses during a period not exceeding 24 hours at different dose levels. Different parameters like clinical signs, mortality and body weight is recorded during the study period. Detailed macroscopic examination is performed for found dead or terminally sacrificed animals. Satellite groups for toxicokinetic may be maintained to determine test item exposure. Study is conducted as per OECD guideline 423 / 425.

2.0 Repeated Dose Toxicity Studies

2.1 4 or 7-Days Repeated Dose Toxicity and Toxicokinetic Study in Rat and Mice

The information obtained from these studies is useful to provide preliminary identification of target organs for toxicity as well as for the dose selection for the long term studies. To evaluate the toxicity of test item, animals are dosed at three to four dose levels for 4 / 7 days. Different parameters like mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, coagulation parameters, urine analysis, organ weights, detailed macroscopic examination and histopathology of major organs is studied. Satellite groups are also maintained to determine toxicokinetic parameters on day 1 and day 4/7.

2.2 14-Days Repeated Dose Toxicity and Toxicokinetic Study in Rat and Mice

To evaluate repeated dose toxicity of test item, animals are dosed at three or four dose levels for 14 days. Different parameters like mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, coagulation

parameters, urine analysis, organ weights, detailed macroscopic examination and histopathology of major organs are studied. Satellite groups are also maintained to determine toxicokinetic parameters on day 1 and day 14. MTD and NOAEL are determined based on the findings.

2.3 28-Days Repeated Dose Toxicity and Toxicokinetic Study in Rat and Mice with Recovery Period

In order to determine sub-acute toxicity of test item, animals are dosed at three or four dose levels for 28 days. Different parameters like mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, coagulation parameters, urine analysis, organ weights, detailed macroscopic examination and histopathology of all organs are studied.

Reversal of test item related effects are also evaluated. Satellite groups are maintained to determine toxicokinetic parameters on day 1 and day 28. MTD and NOAEL are determined based on the findings.

3.0 Genotoxicity Studies

3.1 *In Vitro* Bacterial Reverse Mutation Test - AMES (*Standard & Mini*)

To evaluate the mutagenic potential of any compound, five strains of *Salmonella typhimurium* histidine auxotroph mutants (TA98, TA100, TA102, TA1535 and TA1537) that are deficient in the synthesis of histidine are used. A minimum of 5 concentrations of test item is evaluated using plate incorporation and pre incubation methods in the presence and absence of metabolic activation. By using bacterial strains that carry different mutations, it is possible to determine the type of genetic mutation (e.g. deletion, substitution, etc) induced of a mutagenic compound.

Suven also offers a miniaturized version of the Ames test that requires a small amount of compound compared with the standard test. The Ames assay is performed in 6-well plates using two to five *Salmonella* strains to detect mutagenic potential of test items.

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3.2 *In Vitro* Chromosome Aberration Test in CHO K1 cells or Human Peripheral Blood Lymphocytes

In order to determine clastogenic potential of test item, CHO K1 cells are exposed to test item at various concentrations including negative and positive controls. Cell cycle is arrested in mitosis phase and, chromosomes are evaluated for presence any numeric as well as structural damage.

3.3 *In Vitro* Micronucleus Test in CHO K1 cells

To evaluate genotoxic potential of test item, CHO K1 ells are exposed to test item at various concentrations including negative and positive controls. Cytochalasin treatment is given to arrest the cytokinesis. Binucleated cells are examined and counted for presence of micronuclei.

3.4 *In Vivo* Mammalian Erythrocyte Micronucleus Test in Swiss Albino Mice

To evaluate genotoxic potential of test item, mice are dosed at six dose levels for 2 days including positive and negative controls. Bone marrow of femur bones is harvested and slides are prepared and scored for presence of micronucleus in polychromatic erythrocytes. Test item concentration at different time points are also determined from satellite groups.

3.5 *In Vivo* Chromosome Aberration Test in Rat and Mice

In order to determine genotoxic potential of test item, animals are dosed at six dose levels for 2 days including positive and negative controls. Bone marrow from femur bones is harvested and slides are prepared and evaluated for presence any numeric as well as structural damage in chromosome. Test item concentration at different time points is also determined from satellite groups.

4.0 Method validation for Bio-analysis

LC-MS/MS Method development for quantification of analyte of interest in plasma, urine or tissues and partial validation including linearity, accuracy and precision, recovery, and matrix stability.

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