ACUTE ORAL TOXICITY STUDY (FIXED DOSE METHOD) OF ORYKTA® IN RATS

Purpose:
To determine the toxic potential of the test article by oral ingestion. The study included a sighting study at 300.0 mg/kig, 2000 mg/kg and a limit test at 2000.0 mg/kg of body weight.

Method:
Five female rats were administered orally through a gavage needle Dosed at 2000.0 mg/kg (or a dose volume of 10.0 mL/kg). Individual doses were individually calculated for each animal based on the body weight of the animal.

Animals were observed for a 14 day period after dosing. Body weights were recorded before initiation of the treatment, at Day 7, and at the end of the study (Days 13 and 14).

Summary:
No effects of toxicity or mortalities were observed post dosing and during the 14-day observation period. Animals gained body weight by Day 7 and at the end of the study. No gross pathological findings were observed at necropsy in any of the animals.

DURATION:
14 DAYS

PERFORMED BY NUCRO-TECHNICS INC.

Study complies with OECD Principles of Good Laboratory Practice.

ORYKTA® BACTERIAL REVERSE MUTATION (AMES) TEST

Purpose: To test Orykta® and the metbolites of its components for their potential to induce point mutations in amino acid requiring trains of *Salmonella typhimurrium* and *Escherichia coli*.

Bacteria:
The tester strains were Salmonella typhimurium histidine auxotrophs, TA 98, TA 100, TA 1535, TA 1537 as described by Maron, D.M. and Ames, B.N, et al. (1983). The Escherichia coli strain, WP2 uvrA, is tryptophan- deficient (Brusick, E.J., et al., 1980). All strains were provided as dried discs by Molecular Toxicology Inc. Boone, NC, U.S.A. Frozen permanent stocks were prepared from fresh cultures of the discs with the addition of 9 % dimethysulfoxide and stored at <=-70C.

Controls:
Negative control was sterile distilled DMSO for experiment 1 and H2O for experiment 2.

Positive controls for experiments without S9 were aqueous solutions of sodium azide [CAS no. 26628-22-8] and methylmethanesulfonate [CAS no.66-27-3]; and DMSO solutions of 2-nitroflurene [CAS no.607-57-8], 9-aminoacridine hydrochloride [CAS no. 52417-22-8]. For experiments with S9 mix, benzo[a]pyrene [CAS no. 50-32-8] and 2-aminoanthracene [CAS no. 613-13-8] were dissolved in DMSO; cyclophosphamide [CAS no. 50-18-0], in water.

Conclusion: Orykta® is not mutagenic to S. typhimurium strains TA-98, TA-100, TA-1535, TA-1537 and E. coli strain WP2 uvrA under the experiment conditions.

DURATION:
18 DAYS

PERFORMED BY NUCRO-TECHNICS INC.

The study designed followed the ***OECD Guideline for Testing of Chemicals – 471, Bacterial Reverse Mutation Test***. The conduct of the study complied with the current regulations of ***Good Laboratory Practice***.