charles river

FINAL REPORT

Test Facility Study Code: 31989612

A Bacterial Reverse Mutation Assay of AC-Oxyrase

GLP

SPONSOR:
Oxyrase Incorporated
3000 Park Avenue W,
Ontario, 44906
USA

TEST FACILITY:
Charles River Laboratories Hungary Kft.
H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1.,
Hungary

1. SUMMARY

The test item was tested for potential mutagenic activity using the Bacterial Reverse Mutation Assay.

The experiments were carried out using histidine-requiring auxotroph strains of *Salmonella typhimurium* (*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537) and the tryptophan-requiring auxotroph strain of *Escherichia coli* (*Escherichia coli* WP2 *uvrA*) in the presence and absence of a post mitochondrial supernatant (S9 fraction) prepared from the livers of phenobarbital/β-naphthoflavone-induced rats.

The study included a Preliminary Compatibility Test, a Preliminary Range Finding Test (Plate Incorporation Method), an Assay 1 (Plate Incorporation Method) and an Assay 2 (Pre-Incubation Method).

Based on the available information and Compatibility Test, the test item was formulated in distilled water at a concentration of 50 mg TOS/mL and considered a suitable vehicle. Concentrations of 5000, 2500, 1000, 316, 100, 31.6 and 10 µg TOS/plate were examined in the Preliminary Range Finding Test in *Salmonella typhimurium* TA98 and TA100 tester strains in the absence and presence of metabolic activation. Based on the results of the preliminary experiment, the examined test concentrations in Assay 1 and Assay 2 were 5000, 1581, 500, 158.1, 50 and 15.81 µg TOS/plate.

Moderate precipitate/slight precipitate was detected on the plates in the main tests in all examined bacterial strains with and without metabolic activation at the concentrations of 5000 and 1581 µg TOS/plate.

No inhibitory or toxic effects were observed in the main tests in all examined bacterial strains with and without metabolic activation.

In the assays the number of revertant colonies did not show any biologically relevant increase compared to the solvent controls. There were no reproducible dose-related trends and there was no indication of any treatment-related effect.

The mean values of revertant colonies of the negative (vehicle/solvent) control plates were within the historical control range, the reference mutagens showed the expected increase in the number of revertant colonies and the viability of the bacterial cells was checked by a plating experiment in each test. At least five analyzable concentrations were presented in all strains of the main tests and the examined concentration range was considered to be adequate. The study is considered to be valid.

The reported data of this mutagenicity assay show that under the experimental conditions applied the test item **did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.**

In conclusion, the test item AC-Oxyrase had no mutagenic activity on the growth of the bacterial strains under the test conditions used in this study.

All microscopic findings were regarded as background observations encountered in rats of this age and strain.

24.10. Sperm sample analysis

(Table 12 - Table 14 and Table 21 - Table 22, Appendix 16 - Appendix 18)

Sperm motility, morphology and sperm count in all dosed groups were comparable to controls and to Group 2, there was no test item related effect found.

25. DISCUSSION

No mortality occurred during the study.

No test item related clinical observations were recorded, and no test item related changes were observed in body weight, body weight gain or food consumption throughout the study in the dosed groups.

There was no effect of test item noted during the assessment of grip strength, foot splay, Irwin Test or locomotor activity (LMA) in any dose group when compared to control animals (Group 1, 2).

No abnormalities were recorded during ophthalmic evaluation at pre-test or at the end of the treatment period.

No test item related changes were observed in the measured haematology, coagulation or clinical chemistry parameters. No test item related effect was seen in the measured T3, T4 and TSH thyroid hormone parameters.

There were no test item-related observations in the animal oestrus cycles. There was no effect on testicular pathology.

During pathology investigation, there were no organ weight findings and no macroscopic or microscopic histological changes in organs or tissues related to the test item.

26. CONCLUSION

It is concluded that oral gavage administration of the test item, AC-Oxyrase (as whole cells and fragmented cells) to Wistar rats at dose levels up to 750 mg/kg body weight/day administered for 90 consecutive days was well-tolerated, with no evidence of any adverse finding in the measured in-life parameters. Consequently, the no-observed-adverse-effect level (NOAEL) was considered to be 750 mg/kg body weight/day in case of whole cells and 200 mg/kg body weight/day for fragmented cells.