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FINAL REPORT

Test Facility Study Code: 31989613

An *In Vitro* Mammalian Cell Micronucleus Test of AC-Oxyrase

GLP

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Therefore, concentrations of 100, 50 and 25 µg TOS/mL (a total of three) were chosen for evaluation in case of the short treatment with metabolic activation, concentrations of 300, 150 and 50 µg TOS/mL (a total of three) were chosen for evaluation in case of the short treatment without metabolic activation and concentrations of 75, 25 and 12.5 µg TOS/mL (a total of three) were chosen for evaluation in case of the long treatment without metabolic activation.

The 24-hour treatment without metabolic activation at the concentration of 25 µg TOS/mL caused a statistically significant increase in the number of micronucleated cells when compared to the appropriate negative (vehicle) control at $p<0.05$ level, although the observed values were within the general historical control range of negative (vehicle) control. Therefore, it was considered as biologically not relevant, and this experiment is considered to be negative.

Summarized data are shown in Table 8-Table 10 of Appendix 5.

The Slide Reading Report (micronucleus analysis) is presented in Appendix 7.

24.3 Validity of the Study

The tested concentrations in the *in vitro* micronucleus test were selected based on the results of the preliminary test. Insolubility and cytotoxicity were detected in Assay 1 and Assay 2. The evaluated concentration ranges of the main tests were considered to be adequate, as they covered the range from toxicity to no or little toxicity.

Tree test item concentrations were evaluated in all experiments, this meets the criteria of the OECD No. 487 guideline.

The spontaneous frequency of micronucleated cells of the negative (vehicle) controls in the performed experiments were within the acceptable range.

In the performed experiments, the positive control substances (Cyclophosphamide in the experiment with metabolic activation; Mitomycin C and Colchicine in the experiments without metabolic activation) caused the expected statistically significant increase in the number of micronucleated cells (Table 8-Table 10 of Appendix 5) demonstrating the sensitivity of the test system in each assay.

Historical control data are presented in Appendix 6.

The study is therefore considered to be valid.

25. CONCLUSION

The test item was tested for potential genotoxic activity using the *in vitro* micronucleus test in mammalian cells. The study included a preliminary test for dose selection and two main tests.

The performed experiments were considered to be valid and to reflect the real potential of the test item to cause cytogenetic damage in the cultured mouse lymphoma L5178Y $TK^{+/-}$ 3.7.2 C cells used in this study.

The 24-hour treatment without metabolic activation at the concentration of 25 µg TOS/mL caused a statistically significant increase in the number of micronucleated cells when compared to the appropriate negative (vehicle) control at $p<0.05$ level, although the observed values were within the general historical control range of negative (vehicle) control.

In conclusion, AC-Oxyrase did not cause statistically and biologically significant reproducible increases in the frequency of micronucleated mouse lymphoma L5178Y

$TK^{+/-}$ 3.7.2 C cells in the performed experiments with and without metabolic activation. Therefore, under the conditions of this study, AC-Oxyrase is proved not to be genotoxic.

26. DISTRIBUTION OF THE FINAL REPORT

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27. REFERENCES

1. OECD Guidelines for the Testing of Chemicals, Section 4, Test Guideline No. 487: "*In Vitro* Mammalian Cell Micronucleus Test" (Adopted: 19 July 2016, Corrected: 4 July 2023).
2. Ames, B. N., Joyce McCann and Edith Yamasaki: Methods for Detecting Carcinogens and Mutagens with the *Salmonella* / Mammalian-Microsome Mutagenicity Test. *Mutation Research* (1975) 31, 347-364.
3. Maron, D. M. and Ames, B. N.: Revised Method for the *Salmonella* Mutagenicity Test. *Mutation Research* (1983) 113: 173-215.
4. Hungarian Good Laboratory Practice Regulations: 42/2014. (VIII. 19.) EMMI decree of the Ministry of Human Capacities which corresponds to the OECD GLP, ENV/MC/CHEM(98)17.
5. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1, OECD Principles on Good Laboratory Practice (as revised in 1997), GLP ENV/MC/CHEM(98)17, 1998.