**A bacterial reverse mutation test of Project X**

**SUMMARY AND CONCLUSION**

In order to examine the mutagenic potential of Project X, a reverse mutation assay was conducted in *Salmonella typhimurium* (hereinafter referred to as *S. typhimurium*) TA100, TA1535, TA98 and TA1537, and *Escherichia coli* (hereinafter referred to as *E. coli*) WP2 *uvrA* with and without metabolic activation by the pre-incubation method.

Dimethylsulfoxide (hereinafter referred to as DMSO) was used as the vehicle for the test article.

The dose levels are shown as PROJECT X below.

A dose-finding test was conducted with a total of 10 dose levels (0.254, 0.762, 2.29, 6.86, 20.5, 61.7, 185, 556, 1670 and 5000 μg/plate) after the 50 mg/mL solution was diluted 9 times using a common ratio of 3. In the dose-finding test, growth inhibition by the test article without metabolic activation was observed at 20.5 µg/plate and above for all strains. Growth inhibition with metabolic activation was observed at 61.7 µg/plate and above for *S. typhimurium* TA1535 and TA1537, and at 185 µg/plate and above for *S. typhimurium* TA100 and TA98, and at 1670 µg/plate and above for *E. coli* WP2 *uvrA*. Precipitation of the test article on the plate was observed at 185 µg/plate and above without metabolic activation and at 556 µg/plate and above with metabolic activation. Coloration by the test article in the plate was not observed at any dose level with or without metabolic activation.

Therefore, for the main test without metabolic activation, the dose levels for all tester strains were set at a total of 7 dose levels (0.488, 0.977, 1.95, 3.91, 7.81, 15.6 and 31.3 μg/plate) and 31.3 μg/plate was diluted 6 times using a common ratio of 2. For the main test with metabolic activation, the dose levels for *S. typhimurium* TA1535 and TA1537 were set at a total of 7 dose levels (1.95, 3.91, 7.81, 15.6, 31.3, 62.5 and 125 µg/plate) and 125 μg/plate was diluted 6 times using a common ratio of 2, the dose levels for *S. typhimurium* TA100 and TA98 were set at a total of 7 dose levels (3.91, 7.81, 15.6, 31.3, 62.5, 125 and 250 µg/plate) and 250 μg/plate was diluted 6 times using a common ratio of 2, and the dose levels for *E. coli* WP2 *uvr*A were set at a total of 7 dose levels (31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/plate) and 2000 μg/plate was diluted 6 times using a common ratio of 2.

**Precipitation and Coloration by Test Article**

Precipitation of the test article on the plate was observed at 185 µg/plate and above without metabolic activation and at 500 µg/plate and above with metabolic activation. Coloration by the test article in the plate was not observed at any dose level with or without metabolic activation.

**Growth Inhibition**

Growth inhibition by the test article without metabolic activation was observed at 15.6 µg/plate and above for *S. typhimurium* TA1535, TA98, TA1537 and *E. coli* WP2 *uvr*A, and at 20.5 µg/plate and above for *S. typhimurium* TA100. Growth inhibition by the test article with metabolic activation was observed at 31.3 µg/plate and above for *S. typhimurium* TA1537, and at 61.7 µg/plate and above for *S. typhimurium* TA1535, and at 125 µg/plate and above for *S. typhimurium* TA100 and TA98, and at 1000 µg/plate and above for *E. coli* WP2 *uvr*A.

**Number of Revertant Colonies**

In the dose-finding test and the main test, there was neither test article-related increase in the number of revertant colonies nor dose-response in any test system with or without metabolic activation.

In conclusion, Project X had no bacterial reverse mutagenic activity (negative) under the conditions of this study.