**A Bacterial Reverse Mutation Test of Project 3**

**10 SUMMARY AND CONCLUSION**

In order to assess the potential of Project 3 to induce gene mutation, a bacterial reverse mutation test was performed with 5 strains of bacteria [*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (WP2*uvrA*)], using the pre-incubation method with and without metabolic activation. A vehicle (Dimethyl sulfoxide) and 4 known mutagenic compounds were selected as the negative control and positive control articles, respectively. The dose-finding test and the main test were performed at the following dose levels:

Dose-finding test:

Without and with metabolic activation

5, 15, 50, 150, 500, 1500, and 5000 μg/plate as PROJECT 3 (all test strains)

Main test:

Without and with metabolic activation

78.1, 156, 313, 625, 1250, 2500, and 5000 μg/plate as PROJECT 3 (all test strains)

• In comparison with the negative control, a 2-fold or greater increase in the number of revertant colonies was not observed in any test strain in the dose-finding test or the main test, without or with metabolic activation.

• Growth inhibition in the dose-finding test was observed at 1500 μg/plate and greater in TA98, TA100, TA1535, and TA1537 and at 5000 μg/plate in WP2*uvrA* without metabolic activation, and at 1500 μg/plate and greater in all test strains with metabolic activation. In the main test, growth inhibition was observed at 1250 μg/plate and greater in TA100, TA1535, and TA1537, at 2500 μg/plate and greater in TA98, and at 5000 μg/plate in WP2*uvrA* without metabolic activation, and at 1250 μg/plate and greater in TA100, TA1535, and TA1537 and at 2500 μg/plate and greater in TA98 and WP2*uvrA* with metabolic activation.

• On the plates after incubation for 48 hours, test article precipitation was observed at 5000 μg/plate without and with metabolic activation in the dose-finding test. In the main test, test article precipitation was observed at 2500 μg/plate and greater and 5000 μg/plate without and with metabolic activation, respectively.

• The number of revertant colonies in both the negative and positive controls was within the range (mean ± 3SD) of the background data of SNBL DSR. Accordingly, it was judged that this study was performed satisfactorily.

It was concluded that, under the conditions of this study, Project 3 did not induce gene mutation in bacteria.