**A Bacterial Reverse Mutation Test of PROJECT Y**

**SUMMARY AND CONCLUSION**

In order to assess the potential of PROJECT Y 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.

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 (all strains)

Main test:

Without metabolic activation

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

With metabolic activation

156, 313, 625, 1250, 2500, and 5000 μg/plate (all 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 and main test was observed at 1250 μg/plate and greater in TA100 and TA1535, at 1500 μg/plate and greater in TA98, and at 5000 μg/plate in TA1537 without metabolic activation.

• On the plates after incubation for 48 hours, test article precipitation was observed at 500 μg/plate and greater and at 5000 μg/plate without and with metabolic activation, respectively in the dose-finding test. In the main test, test article precipitation was observed at 625 μg/plate and greater and at 2500 μg/plate and greater, 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 Y did not induce gene mutation in bacteria.