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

**10 SUMMARY AND CONCLUSION**

In order to assess the potential of Project S 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 preincubation method without and with 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

1.5, 5, 15, 50, 150, 500, 1500, and 5000 μg/plate as PROJECT S (all test strains)

Main test:

Without and with metabolic activation

31.3, 62.5, 125, 250, 500, 1000, and 2000 μg/plate as PROJECT S (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 all test strains without and with metabolic activation. In the main test, growth inhibition was observed at 1000 μg/plate and greater in TA98, TA100, TA1535, and TA1537 and at 2000 μg/plate in WP2*uvrA* without and with metabolic activation.

● On the plates after incubation for 48 hours in the dose-finding test, test article precipitation was observed at 5000 μg/plate with metabolic activation. In the main test, test article precipitation was not observed at up to 2000 μg/plate without or with metabolic activation.

● 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 S did not induce

gene mutation in bacteria.