**A Bacterial Reverse Mutation Test of PROJECT 2**

**SUMMARY AND CONCLUSION**

In order to assess the potential of PROJECT 2 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: (all strains)

With and without metabolic activation

5, 15, 50, 150, 500, 1500, and 5000 μg/plate

Main test: (all strains)

Without metabolic activation

39.1, 78.1, 156, 313, 625, 1250, and 2500 μg/plate (TA98 and WP2*uvr*A)

19.5, 39.1, 78.1, 156, 313, 625, and 1250 μg/plate (TA100, TA1535, and TA1537)

With metabolic activation

19.5, 39.1, 78.1, 156, 313, 625, and 1250 μg/plate (TA98, TA100, TA1535, and TA1537)

78.1, 156, 313, 625, 1250, 2500, and 5000 μg/plate (WP2*uvr*A)

1. 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, with or without metabolic activation.

2. Growth inhibition was observed at 500 μg/plate and greater in TA98, TA100, TA1535 and TA1537, and at 5000 μg/plate in WP2*uvr*A with metabolic activation, and at 1250 μg/plate and greater in TA98 and WP2*uvr*A, at 500 μg/plate and greater in TA100 and TA1535, and at 625 μg/plate and greater in TA1537 without metabolic activation.

3. Upon addition of the test article preparation, test article precipitation was observed at 625 μg/plate and greater and at 5000 μg/plate, with and without metabolic activation, respectively. On the plates after incubation for 48 hours, test article precipitation was observed at 625 μg/plate and greater with metabolic activation. Test article precipitation was not observed up to 5000 μg/plate on the plates after incubation for 48 hours without metabolic activation.

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

It was concluded that PROJECT 2 did not induce gene mutation in bacteria when tested under the conditions of this study.