**A Bacterial Reverse Mutation Test of Project 4**

**SUMMARY AND CONCLUSION**

In order to assess the potential of Project 4 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 (WP2uvrA)], 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 as PROJECT 4

Main test:

With metabolic activation

39.1, 78.1, 156, 313, 625, 1250, and 2500 µg/plate as PROJECT 4

(TA100, TA1535, and TA1537) 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate as PROJECT 4

(TA98, and WP2uvrA)

Without metabolic activation

39.1, 78.1, 156, 313, 625, 1250, and 2500 µg/plate as PROJECT 4

(TA98, TA100, TA1535, and TA1537) 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate as PROJECT 4

(WP2uvrA)

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 1500 µg/plate and greater in TA100, TA1535, and TA1537, and at 5000 µg/plate in TA98 and WP2uvrA, with metabolic activation, and at 1500 µg/plate and greater in TA98, TA100, TA1535, and TA1537, and at 5000 µg/plate in WP2uvrA, without metabolic activation.

3. Test article precipitation was not observed up to 5000 µg/plate upon addition of the test article formulation, or on the plates after incubation for 48 hours, with or 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 4 did not induce gene mutation in bacteria when tested under the conditions of this study.