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

**10 SUMMARY AND CONCLUSION**

The objective of this study was to assess the potential of Project D for inducibility

of gene mutation.

A bacterial reverse mutation test was performed with 5 test strains of bacteria [*Salmonella typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (WP2*uvrA*)], using the pre-incubation method with and without metabolic activation. Based on the results of the dose-finding test at 1.5, 5, 15, 50, 150, 500, 1500, and 5000 μg/plate as PROJECT D with and without metabolic activation, the main test was performed at 3.91, 7.81, 15.6, 31.3, 62.5, 125, and 250 μg/plate as PROJECT D in TA100, TA1535, WP2*uvrA*, and TA98, and at 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, and 125 μg/plate as PROJECT D in TA1537 without metabolic activation, and at 3.91, 7.81, 15.6, 31.3, 62.5, 125, and 250 μg/plate as PROJECT D in TA100, TA1535, and TA98, and at 7.81, 15.6, 31.3, 62.5, 125, 250, and 500 μg/plate as PROJECT D in WP2*uvrA*, and at 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, and 125 μg/plate as PROJECT D in TA1537 with metabolic activation.

Test article precipitation was observed at 125 μg/plate and greater without metabolic activation and 250 μg/plate and greater with metabolic activation upon addition of the test article formulation, and 250 μg/plate and greater without metabolic activation and 500 μg/plate and greater with metabolic activation on the plates after incubation for 48 hours. Growth inhibition was observed at 125 μg/plate and greater in all test strains without metabolic activation, and at 125 μg/plate and greater in TA1537, and at 150 μg/plate and greater in TA100, TA1535, and TA98, and at 250 μg/plate and greater in WP2*uvrA* with metabolic activation.

In comparison with the negative control, no 2-fold or greater increase in the number of revertant colonies was observed in any test strain with or without metabolic activation.

It was concluded that Project D has no potential to induce gene mutation in bacteria under the conditions of this study.