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

**10 SUMMARY AND CONCLUSION**

In order to assess the potential of Project R 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

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

Main test:

Without metabolic activation

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

156, 313, 625, 1250, 2500, and 5000 μg/plate as PROJECT R (WP2*uvrA*)

With metabolic activation

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

156, 313, 625, 1250, 2500, and 5000 μg/plate as PROJECT R (TA1535 and WP2*uvrA*)

● 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 500 μg/plate and greater in TA98, TA100, TA1535, and TA1537 without metabolic activation, and at 5000 μg/plate in TA98, TA100, and TA1537 with metabolic activation. In the main test, growth inhibition was observed at 313 μg/plate and greater in TA1537, at 625 μg/plate and greater in TA98 and TA1535, and at 1250 μg/plate in TA100 without metabolic activation, and at 2500 μg/plate and greater in TA100 and TA1537, and at 5000 μg/plate in TA98 with metabolic activation. Growth inhibition was not observed in TA1535 with metabolic activation or WP2*uvrA* without or with metabolic activation, in the dose finding test or the main test.

● On the plates after incubation for 48 hours in the dose-finding test, test article precipitation was observed at 500 μg/plate and greater and at 1500 μg/plate and greater without and with metabolic activation, respectively. In the main test, test article precipitation was observed at 313 μg/plate and greater and at 1250 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 R did not induce gene mutation in bacteria.