**Four-Week Repeated Oral Dose Toxicity Study of Project K in Dogs Followed by a 4-Week Recovery Period**

**11 SUMMARY**

Project K suspended in 0.5% methylcellulose solution was orally administered to 4 male and 4 female beagle dogs per group at doses of 0.03, 0.3, and 100 mg/kg as PROJECT K once daily for 4 wk to evaluate toxicological changes. A control group received 0.5% methylcellulose solution, the vehicle for the test article.

A recovery group consisting of 3 male and 3 female beagle dogs treated with 100 mg/kg was also included, to investigate whether toxic changes induced during 4 wk of treatment period resolved after a 4-wk recovery period.

To evaluate systemic exposure, the plasma PROJECT K concentration was also measured.

During the treatment period, animals underwent observation of general signs, measurement of body weight and food consumption, hematology, blood chemistry testing, urinalysis, ophthalmologic examination, electrocardiography, and toxicokinetic analysis. During the recovery period, animals underwent observation of general signs, measurement of body weight and food consumption, hematology, blood chemistry testing, and urinalysis. Gross pathological examination, organ weight measurement, and histopathological examination were conducted at the completion of the treatment and recovery periods.

There was no animal death in any dose group.

Toxicological changes were not observed at 0.03 mg/kg, but did occur at 0.3 mg/kg and more.

The main findings are described below (Appendix 1).

At 0.3 mg/kg and more, findings included vomiting, soft feces, and abnormal-colored feces (reddish, reddish soft, reddish watery, and reddish mucous feces; dark-reddish, dark-reddish soft, dark-reddish watery, and dark-reddish mucous feces; with positive occult blood test); increases in fibrinogen; and decreases in total protein, albumin, calcium, and sodium; and slight infiltration of inflammatory cells in the duodenal mucosa.

At 100 mg/kg, findings included those observed in the 0.3 mg/kg group, as well as the following: vomitus with test article, watery feces, and emaciation; decreases in body weight and food consumption; decreases in RBC count, hemoglobin, hematocrit, mean cell hemoglobin concentration, globulin, albumin/globulin ratio, total bilirubin, total cholesterol, ALP activity, ALT activity, and glucose; and increases in platelet count, reticulocyte percentage, WBC count, neutrophil count, monocyte count, and chlorine. Autopsy findings included depressed focus and dark red focus in the duodenum, ascites retention, edema of the pancreas, and obscure thymus. There were statistically significant increases in absolute and relative weights of the spleen, and relative weight of the pancreas, and a statistically significant difference in thymus weight as compared with the control group. In histopathology, findings were ulcer/erosion of the duodenum; ulcer/erosion and regeneration of mucosal epithelia of the ileum; hypercellularity in sternal and femoral bone marrows; extramedullary hematopoiesis in the spleen; thymic atrophy; granuloma of the liver; and interstitial edema of the pancreas.

In the recovery group, among the changes induced during the 4-wk administration period, increases in platelet count and reticulocyte percentage, and decreased mean corpuscular hemoglobin concentration were still observed. In addition, some animals had decreases in mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. These changes were judged to be secondary to recovery of erythrocyte parameters that decreased or tended to decrease during the treatment period, and were expected to resolve by prolongation of the recovery period since the erythrocyte parameters returned to normal values by 4-wk drug withdrawal. Increased platelet count resolved in 1 of the 2 animals after drug withdrawal.

Soft feces, watery feces, and abnormal-colored feces, increased relative weight of the spleen, and hypercellularity in sternal and femoral bone marrows were alleviated in frequency or severity, and thus they were considered to show tendency to recovery.

No other changes occurred in the recovery group.

When the dose was increased from 0.03 to 0.3 mg/kg, Cmax and AUC24 values on day 1 of males increased at a rate equal to the dose ratio (Cmax, 9.8-fold; AUC24, 9.6-fold) and those of females increased at a rate lower than the dose ratio (Cmax, 7.1-fold; AUC24, 7.5-fold).

When the dose was increased from 0.3 to 100 mg/kg, the values for both males and females increased at a rate lower than the dose ratio (Cmax, 12.6- and 17.0-fold, respectively; AUC24, 24.2- and 17.2-fold, respectively).

Comparison of the Cmax and AUC24 values between day 1 and week 2 or 4 indicated that the AUC24 value for females in the 100 mg/kg group at week 2 was 2.5 times higher than that on day 1. No other significant difference was observed in any group.

No gender difference was noted.

In summary, the no-observed-adverse-effect level (NOAEL) for this study was judged to be 0.03 mg/kg/day (Appendix 1). Changes during administration can be expected to disappear or tend to disappear by drug withdrawal.