**A 4-Week Repeated Oral Dose Toxicity Study of PROJECT M in Dogs Followed by a 4-Week Reversibility Study**

**11 SUMMARY**

PROJECT M was suspended in 0.5 w/v% methylcellulose solution and administered orally once daily for 4 weeks at dose levels of 0 (vehicle), 3, 10, 30, and 100 mg/kg to 4 male and 4 female beagle dogs per group in order to investigate its toxicity. Three males and three females were added to the 30 and 100 mg/kg groups in order to assess the reversibility of toxicity during a subsequent 4-week recovery period. Systemic exposure to PROJECT M was also assessed. The following observations and examinations were performed in this study: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, gross pathology, organ weights, and histopathology.

In the 100 mg/kg group, 1 female died on Day 7 of dosing, 1 female was sacrificed due to moribundity on Day 13 of dosing, and 1 male died on Day 6 of recovery.

In the female that died before dosing on Day 7, food consumption was notably decreased on 2 days before death, and abnormal stool color (reddish brown, positive occult blood reaction), mucous stool, lateral position, and hemorrhage from the lung (red-colored vomiting, and hemorrhage in the lung in histopathologic examination and red focus in the lung, and red foamy fluid in the trachea and bronchus at gross pathology) were observed before death. In addition, clonic convulsions 5 times, and tonic convulsion were observed just before death.

Test article-related changes were observed in the liver and digestive tract. Hypertrophy and cytoplasmic vacuolation of hepatocyte, which were positive for oil red O staining, focal mucosal hemorrhage in the ileum, colon, and rectum were observed in histopathologic examination. Vomiting was observed during the dosing period.

In the moribund sacrificed female before dosing on Day 13 of dosing, food consumption was notably decreased on 3 days before sacrifice, and lateral position was observed before sacrifice, and it was euthanized due to moribundity. Test article-related changes were observed in the liver, digestive tract, and kidney. Hypertrophy and cytoplasmic vacuolation of hepatocyte, which were positive for oil red O staining, degeneration and necrosis of centrilobular hepatocyte, and centrilobular hemorrhage in histopathologic examination, high liver weights were noted, and increased aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, total bile acid, and lactate dehydrogenase were noted in blood chemical examination. In addition, focal mucosal hemorrhage in the stomach and jejunum, and dilatation of renal tubule and vacuolation of the tubular epithelium, which were positive for oil red O staining in histopathologic examination, high kidney weights were noted, and increased blood urea nitrogen and creatinine were also noted in blood chemical examination. Increased erythrocyte count, hematocrit value, hemoglobin concentration, monocyte count, albumin, and inorganic phosphorus, and decreased eosinophil count, lymphocyte count, fibrinogen, glucose, potassium, and chloride, and prolonged prothrombin time and activated partial thromboplastin time were noted in hematological and blood chemical examinations when the animal was sacrificed moribund. Vomiting was observed during the dosing period.

In the male that died on Day 6 of recovery, food consumption was notably decreased from Day 26 of dosing to the day of death, and lateral position was observed on Day 28 of dosing, and decrease in spontaneous activity was observed from Day 28 of dosing to the day of death, and lateral position, gasping, and vomiting were observed just before death. At gross pathology, retention of white foamy fluid in trachea and bronchus was observed. Test article- related changes were observed in the liver, and digestive tract. Foreign body in the bronchus and bronchiole, alveolus with hemorrhage in the lung, and mucosal atrophy of the esophagus, stomach, and duodenum, erosion and mucosal fibrosis in the jejunum, ileum, cecum, and rectum, degeneration and necrosis of centrilobular hepatocyte were observed in histopathologic examination, and increased alkaline phosphatase and total bilirubin were noted in blood chemical examination. Decreased erythrocyte count, hematocrit value, hemoglobin concentration, eosinophil count, and lymphocyte count, and increased large unstained cells count, fibrinogen, and total cholesterol had been noted in hematological and blood chemical examinations on Day 27 of dosing. Vomiting and salivation were observed during the dosing period.

No treatment-related changes were noted in males or females in the 3 or 10 mg/kg group, or in males in the 30 mg/kg group during the dosing or recovery period.

In 1 female in the 30 mg/kg group, test article-related changes were observed in the digestive tract and liver. Erosion, diffuse mucosal inflammation and regeneration of the epithelium in the ileum, and hypertrophy and cytoplasmic vacuolation of hepatocyte in the liver, which were positive for oil red O staining, degeneration and necrosis of centrilobular hepatocyte and centrilobular hemorrhage were observed in histopathologic examination as well as in died or moribund sacrificed animals. These changes were not observed in animals of the recovery group of 30 mg/kg.

In the surviving animals in the 100 mg/kg group, decrease in spontaneous activity was observed in 1 female. Vomiting and salivation were observed, and decreased food consumption and body weight were noted in males and females during the dosing period. Test article-related changes were observed in the digestive tract and kidney. Diffuse mucosal inflammation in the stomach, and vacuolation of the tubular epithelium, which was positive for oil red O staining in the kidney in histopathologic examination were each observed in 1 female. Decreased sodium and chloride in urinalysis, decreased eosinophil count, albumin, glucose, calcium, and chloride, and increased platelet count, monocyte count, large unstained cells count, fibrinogen, alanine aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, total bile acid, total cholesterol, and triglycerides were noted in hematological and blood chemical examinations, and high liver weight were noted in females at Week 4 of dosing. Those changes were not noted in the recovery period.

In toxicokinetics, the mean tmax values on each dosing day were 0.5 to 0.6 hours, 0.6 to 0.9 hours, 0.6 to 1.0 hours, and 0.9 to 1.7 hours at 3, 10, 30, and 100 mg/kg, respectively. In males, the mean Cmax values increased almost dose-proportionally between 3 and 30 mg/kg and less than dose-proportionally between 30 and 100 mg/kg. The mean AUC24 values increased more than dose-proportionally between 3 and 30 mg/kg and there was marked increase between 10 and 30 mg/kg, and less than dose-proportionally between 30 and 100 mg/kg. In females, the mean Cmax values also increased almost dose-proportionally between 3 and 30 mg/kg and less than dose-proportionally between 30 and 100 mg/kg. The mean AUC24 values increased more than dose-proportionally between 3 and 10 mg/kg/day, almost dose-proportionally between 10 and 30 mg/kg/day, and less than dose-proportionally between 30 and 100 mg/kg. For all dosing groups and dosing days, no sex difference was observed in the TK parameters. In males, the mean AUC24 values at 10, 30, and 100 mg/kg and mean Cmax values at 30 mg/kg were highest on Day 28, followed by Day 14 and Day 1 in order, and the mean AUC24 values at 3 mg/kg dosing, and mean Cmax values at 3, 10, and 100 mg/kg groups didn’t show any influence of repeat dosing. In females, the Cmax and AUC24 values of all dosing groups showed no influence of repeat dosing.

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level of was 30 mg/kg/day for males and 10 mg/kg/day for females. During the recovery period, general condition deteriorated in 1 male in the 100 mg/kg group, and this male died, and reversibility in clinical examination could not be evaluated in this male. All changes noted during the dosing period in clinical sign and clinical examination recovered, and treatment- related histopathological findings were not observed at the end of the recovery period.