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

**11 SUMMARY**

Project J was suspended in 0.5 w/v% methylcellulose solution and orally administered once daily for 4 weeks at dose levels of 0 (vehicle control), 1, 3, 30, and 300 mg/kg/day 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 300 mg/kg group to assess the reversibility of toxicity observed during the dosing period in a subsequent 4-week recovery period. Dosing at 300 mg/kg was discontinued for males and females after Day 24 of dosing, and 1 male and 3 females were necropsied at the end of dosing. Three males and three females were necropsied after a recovery period of 32 days.

The following observations and examinations were performed in this study: clinical signs, body weight, food consumption, body temperature, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, gross pathology, organ weights, histopathology, toxicokinetics, and testosterone analysis.

One male died in the morning on Day 24 of dosing, and 2 males and 1 female were sacrificed due to moribundity on Days 15, 17, and 24 of dosing at 300 mg/kg. In these moribund animals and animals that died, decreased spontaneous activity, abnormal position (prone position or lateral position), suppressed response to stimulation, emaciation, no stool, or decreased body weight or food consumption was noted before death or sacrifice.

No toxicological changes were noted at 1 or 3 mg/kg.

At 30 mg/kg, vomiting, decreased serum testosterone concentrations, and atrophy of the prostate were noted in males, and decreased body weight was noted in females.

In the survived animals at 300 mg/kg, decreased spontaneous activity, and decreased body weight or food consumption were noted in males and females. In survived animals, animals that died, and/or moribund animals at 300 mg/kg, soft stool, abnormal stool color (reddish brown or dark red color and positive occult blood reactions) mainly from Week 2 of dosing, and vomiting from Week 1 of dosing, mononuclear cell infiltration, mucosal erosion and hemorrhage in the duodenum, or related gross pathological changes of the duodenum was noted in males and females. Hypocellularity in the sternal and femoral bone marrow, and, atrophy of the mesenteric lymph node, thymus, and Peyer’s patch, and decrease of lipid and hypertrophy in the adrenal zona fasciculata cell were observed, and related gross pathological changes in the adrenal and organ weights of the adrenal were noted, and high spleen weight was noted, and decreased sodium and chloride in urine, decreased erythrocyte count, hemoglobin concentration, and hematocrit value, platelet count, leukocyte count, lymphocyte count, eosinophil count, basophil count, neutrophil count, monocyte count, albumin, albumin/globulin ratio, calcium, sodium, potassium, and chloride, increased neutrophil count, total protein, total cholesterol, glucose, urea nitrogen, creatinine, or prolonged prothrombin time and activated partial thromboplastin time was noted in males and females. Decreased serum testosterone concentrations was noted, and atrophy of the prostate were observed in male. Atrophy of the hepatocyte, single cell necrosis of the hepatocyte, or inflammatory cell infiltration in the liver, or high liver weight was noted, or increased aspartate transaminase, alanine transaminase, alkaline phosphatase, or total bilirubin was noted in males and females. Inflammation and hemorrhage in the lung was noted, and related gross pathological change was noted in males and females.

Additionally, in animals that died or moribund animals at 300 mg/kg, diarrhea at Week 4 of dosing and mucous stool from Week 2 of dosing were observed in males, crypt abscess in the duodenum, mucosal congestion in the jejunum and cecum, neutrophil cell infiltration and regeneration of glandular epithelium in the rectum, follicular atrophy in the spleen, ductal dilatation of the sublingual gland, or atrophy of muscle fiber in the femoral skeletal muscle, multifocal necrosis of the hepatocyte was observed in males and females, or related gross pathological changes in the jejunum was observed in males. Low prostate weight, single cell necrosis of ductal epithelium of the epididymis, spermatid giant cell formation in the testis was observed in males. Decreased heart rate and prolonged QTc were noted in 1 female on Day 22 of dosing.

No test article-related changes were observed in body temperature or ophthalmology at any dose level.

In toxicokinetics, during the dosing period, mean Cmax and mean AUC24 values of both sexes increased with the increasing dose level, and these values increased less than dose- proportionally at 300 mg/kg. Those values at Day 14 and 28 were slightly higher than those at Day 1 in both sexes. During the dosing period, consistent sexual difference in TK parameters was not observed at any doses. The tmax values showed a tendency to be delayed with increase of the dose.

All changes observed at 300 mg/kg during the dosing period recovered or were showed tendency to recover during the 4-week recovery period.

It was concluded that, under the conditions of this study, the NOAEL was 3 mg/kg/day. The changes observed during the dosing period recovered or tended to recover during the 4-week recovery period.