**A 4-Week Oral Dose Toxicity Study of Project L in Beagle Dogs with a 4-Week Recovery Period**

**11 SUMMARY**

Project L was dissolved in 0.5 w/v% methylcellulose solution and orally administered once daily for 4 weeks at dose levels of 0 (vehicle control), 1.5, 5, and 15 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 5 and 15 mg/kg group to assess the reversibility of toxicity observed, and recovery period was initially set for 4 week.

One male and two females were sacrificed due to moribundity on Days 16, 10, and 16 of dosing, respectively at 15 mg/kg. Dosing at 15 mg/kg was discontinued for males after Day 20 of dosing and for females after Day 17 of dosing, and 3 males and 2 females were necropsied at the end of dosing. Three males were necropsied after a recovery period of 36 days, and 3 females were necropsied after a recovery period of 39 days.

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, histopathology, electron microscopy and toxicokinetics.

At 1.5 mg/kg and more, decreased erythrocyte count, hematocrit value, and hemoglobin concentration were noted in males and females. Vomiting, prolonged activated partial thromboplastin time (APTT), decreased albumin, and increased alkaline phosphatase (ALP) were noted in males at 1.5 mg/kg, and males and females at 5 mg/kg or more.

At 5 mg/kg and more, erythrocyte in the sinus in the mesenteric lymph node was noted in males and females. Decreased platelet count, total protein, and albumin/globulin ratio, increased serum chloride, and thickening of the cartilage in the sternum in males at 5 mg/kg, and males and females at 15 mg/kg were noted. Inflammatory cell infiltration or hemorrhage in the lung accompanying gross lesions (red or dark red focus, or red or dark red discoloration in the lung) in females was noted at 5 mg/kg and more.

At 15 mg/kg, clinical signs suggesting deteriorated conditions were noted in males and females in moribund animals and surviving animals. Decreased spontaneous activity, no stool, or decreased body weight or food consumption was noted in surviving animals. In addition, prone position, tachypnea, panting, or suppression of touch response was noted in the male and females in moribund animals. In males and females in moribund animals and surviving animals, soft stool, diarrhea, mucous stool, and abnormal stool color with positive occult blood reactions were observed. Abnormal mucosa (abnormal color, maxillary oral mucosa or tongue), eye mucus, reddish conjunctiva, reddish oral mucosa, and interdigital inflammation, and histopathological lesions of mucosal or epithelial damages including atrophy, erosion, ulcer, or inflammatory cell infiltration in the tongue, eyeball (epithelium in the conjunctiva and cornea), eyelid, skin, and oral mucosa were observed. Decreased lymphocyte count, eosinophil count, total protein, and calcium, and increased monocyte count, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, triglycerides, total cholesterol, urea nitrogen, creatinine, potassium and chloride were noted. Vacuolation or accumulation of foam cells in various organs and tissues [heart, tongue, esophagus, stomach, duodenum, ileum, cecum, rectum, liver, kidney, urinary bladder, eyeball (epithelium in the conjunctiva/cornea/limbus), skin, eyelid, and oral mucosa] were observed. Increased lymphocyte vacuolation ratio in the peripheral blood was noted. In electron microscopy of the liver and kidney, intracytoplasmic single membrane-bound lysosome-like structure in the cytoplasm of Kupffer cells and epithelium of the proximal tubules, corresponding to vacuolation of the Kupffer cells and epithelium in the proximal tubules) in gross lesions were observed. Hemorrhage in the mucosa (lamina propria) or submucosa in the jejunum and gallbladder, atrophy of the mucosa in the ileum, atrophy of lymphoid tissue of the rectum, dilatation of the crypts in the rectum were observed. Atrophy of the spleen, mesenteric lymph node, Peyer's patch, and a decrease in zymogen granules in the acini in the pancreas was observed. Focal interstitial hemorrhage, and basophilic changes in the proximal tubules in the kidneys were observed. Other test article-related lesions were observed (congestion in the spleen, erythrocytes in the sinus and histiocytosis in the sinus in the submandibular lymph nodes, histiocytosis in the sinus in the mesenteric lymph nodes, and hemorrhage in the adrenals). Additionally, high adrenal, spleen, lung, liver, and kidney weights, and gross pathological changes (red discoloration in the conjunctiva, discoloration in the oral mucosa) were noted.

In males, erosion (left buttocks) was observed, and opacity in the cornea in the bilateral eyes was observed, increased large unstained cell count and serum glucose were noted.

Vacuolation in the prostate, hemorrhage in the mucosa in the ileum, atrophy of lymphoid tissue of the cecum and rectum, decrease in the trabecular bone in the sternum, hemorrhage in the myocardium were observed. Additionally, gross pathological changes (swelling of interdigit in the hindlimb, and red or dark red focus, or red discoloration in the kidney, jejunum, ileum, and gallbladder) were observed.

In females, trace of reddish rhinorrhea was observed, increased glucose in urinalysis, and increased globulin, and decreased sodium were noted. Increased erythrocyte count, hematocrit value, and hemoglobin concentration were noted at moribundity. Atrophy of esophageal gland in the esophagus, accumulation of foam cells in the jejunum, vacuolation in the uterus and mammary gland, hemorrhage in the mucosa in the stomach, cecum, ulcer in the stomach, cell debris in the crypts in the cecum and colon, dilatation of the crypts in the colon, mineralization in the vascular wall of the heart were observed, and indicated intracytoplasmic autophagosome-like structures in the Kupffer cells in electron microscopy of the liver were observed. Additionally, high relative heart weight, and gross pathological changes (swelling of interdigit in the forelimb, discoloration in the tongue, enlargement in the spleen, and black discoloration in the stomach and jejunum) were noted.

In toxicokinetics, during the dosing period except Day 28 of 15 mg/kg group, Cmax and AUC24 values of both sexes increased almost dose-proportionally up to 15 mg/kg, and those values at Day 28 were slightly higher than Day 1 and almost same levels at Day 14. There was no appreciable sex difference.

After a 4-week recovery period, inflammatory cell infiltration and hemorrhage in the alveoli in the lung accompanying gross lesion had recovered at 15 mg/kg, although these changes were still observed in 1 female at 5 mg/kg. Additionally, edema in the alveoli and thickening of the pleura were observed in this female at 5 mg/kg, but these were not noted at 15 mg/kg.

At 15 mg/kg, opacity in the cornea observed during the dosing period in 1 male, did not worsen or recover as compared to the dosing period. Other changes recovered or decreased in severity and/or incidence as compared to the interim death or the end of dosing period.

It was concluded that, under the conditions of this study, the NOAEL was less than 1.5 mg/kg/day for males and females. The changes observed during the dosing period recovered or tended to recover during the 4-week recovery period, except for opacity in the cornea in 1 male at 15 mg/kg.