**Final Report**

**A 4-Week Repeated Oral Dose Toxicity Study of PROJECT Q in Dogs**

**Followed by a 4-Week Reversibility Study**

**11 SUMMARY**

PROJECT Q was administered orally once daily for 4 weeks at dose levels of 0 (vehicle: 5% TC-5,R solution), 20, 100, and 600 mg/kg/day to 4 male and 4 female beagle dogs per group in order to investigate its potential toxicity. Three males and three females were added to the 600 mg/kg group in order to assess the reversibility of toxicity during a subsequent 4-week recovery period. Because changes in clinical signs (decrease in spontaneous activity, incomplete eyelid opening, and/or somnolence) and decreases in food consumption and body weight were observed in some males in the 600 mg/kg group, the high dose level was decreased to 300 mg/kg for males from Day 9 of dosing. Systemic exposure to PROJECT Q was also assessed.

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, and toxicokinetics.

At 600 mg/kg, lateral position and abnormal stool color (dark red) were observed before dosing on Day 28 of dosing in 1 female, and therefore this female was euthanized due to moribundity. In this animal, leukocyte and neutrophil counts increased, and prothrombin time (PT) and activated partial thromboplastin time (APTT) prolonged in hematology on Day 28 of dosing, and globulin, glucose, calcium (Ca), and potassium (K) increased in blood chemistry on Day 28 of dosing. In histopathology, atrophy of acinar cell in the submandibular gland, decrease in glycogen in hepatocyte, and erosions in the ileum, colon, and rectum were observed. Other than that, this animal showed similar changes to the other females of this group, including body weight, food consumption, clinical signs, body temperature, urinalysis, electrocardiography, organ weight and histopathology.

At 600 or 600→300 mg/kg, marked decreases in food consumption were observed, and body weight decreased in both sexes. However, in males after reduction of the dose level to 300 mg/kg (Day 9 of dosing), food consumption and body weight recovered, except in 1 male, in which body weight at the end of the dosing period was 78% of the pre-dose value. In females in which body weight decreased, the body weights at the end of the dosing period were 63% to 81% of the pre-dose values. Incomplete eyelid opening and a decrease in spontaneous activity were observed during the dosing period in both sexes. Somnolence was observed in both sexes mainly at Weeks 2 and/or 3 of dosing. Salivation was observed in females from Day 14 or 19 of dosing. Vomiting was observed in both sexes sporadically during the dosing period. Scratching and trauma was observed in males. Body temperature decreased in both sexes on Days 1 and 8 of dosing, and in 1 female 24 hours after dosing on Day 26 of dosing.

At 600 or 600→300 mg/kg, QTc and QRS duration prolonged in females on Day 26 of dosing. In urinalysis, occult blood was observed, specific gravity decreased, and unclassifiable crystals in urinary sediments were observed in both sexes. Total excretions of sodium (Na), chloride (Cl), and/or K decreased in females. In hematology, erythrocyte count, hematocrit value, and hemoglobin concentration decreased in both sexes, while these parameters increased in 2 females on Day 28 of dosing. In both sexes, platelet count decreased, reticulocyte ratio increased, eosinophil count increased, and lymphocyte count decreased on Days 15 and/or 28 of dosing. In blood chemistry, in both sexes, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, and total cholesterol increased on Days 15 and/or 28 of dosing. In females, total bilirubin, total protein, albumin, and/or inorganic phosphorus (IP) increased, and Na and/or Cl decreased on Days 15 and/or 28 of dosing.

At 600 or 600→300 mg/kg, in gross pathology, multiple white foci in the left kidney were observed in 1 female. In organ weights, high kidney weights in females (including the female euthanized due to moribundity) and 1 male, high lung weights in females, and low testis weight in 1 male were observed. High adrenal weights were also observed in females. In histopathology, crystal deposition was observed in giant cell in the lymphatic tissues (submandibular and mesenteric lymph nodes, and Peyer’s patch), lung, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, prostate, and/or skin, in Kupffer cell in the liver, and/or in renal papilla/ureter and renal tubule of the kidney in both sexes. In the kidney, focal dilatation of the renal tubule with mononuclear cell infiltration and/or neutrophil infiltration, focal fibrosis, foreign body granuloma, hemorrhage, hypertrophy in the transitional epithelium and/or regeneration of renal tubule were observed in both sexes. Atrophy and degeneration of the seminiferous tubule in the testis with absence of sperm or cell debris in the epididymis, and immaturity in the prostate were observed in 1 male. Atrophy of the thymus was observed in both sexes. Hyperplasia of the bile duct and atrophy of the pancreatic

acinar cell were observed in females.

At 100 mg/kg, food consumption decreased sporadically in females at Week 1 of dosing, and body weight decreased in 1 female at Week 1 of dosing. Vomiting was observed in both sexes mainly at Weeks 1 and 2 of dosing. Scratching and trauma were observed in 1 male. Body temperature decreased in 1 male 4 hours after dosing on Day 1 of dosing. In urinalysis, specific gravity decreased in both sexes, total excretion of Cl decreased in females, and unclassifiable crystals in urinary sediment were observed in both sexes. In hematology, reticulocyte ratio increased in 1 male on Day 15 of dosing, and APTT prolonged in 1 male on Day 28 of dosing. In blood chemistry, ALP and total cholesterol increased in both sexes on Days 15 and/or 28 of dosing. In histopathology, atrophy and degeneration of the seminiferous tubule in the testis with absence of sperm or cell debris in the epididymis were observed in males.

At 20 mg/kg, vomiting was observed sporadically in 1 female during the dosing period; however, this was not considered toxicologically significant since it was observed in only 1 female.

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

The changes observed at 600 or 600→300 mg/kg during the dosing period recovered or tended to recover during the 4-week recovery period. However, the following changes were observed at the end of the recovery period: decreased specific gravity in urinalysis, and pathological changes, observed as gross abnormalities in the kidney (calculus in the bilateral kidneys, red focus and dilatation of the renal pelvis in the left kidney, and dilatation and calculus in the left ureter) in female, high kidney weight and histopathological changes such as crystal deposition in various tissues in both sexes, and changes in the kidney in females (focal dilatation of the renal tubule with mononuclear cell infiltration and neutrophil infiltration, focal fibrosis, desquamation and hypertrophy in the transitional epithelium, and regeneration of the renal tubule in the kidney, and desquamation of the transitional epithelium in the ureter), and testis and epididymis in males (atrophy and degeneration of the seminiferous tubule in the testis with absence of sperm in the epididymis).

In toxicokinetics, on Day 1 of dosing, mean Cmax and AUC24 increased with dose level in both sexes. Following repeated dosing, in both sexes at 20 and 100 mg/kg and in females at 600 mg/kg, mean Cmax and AUC24 on Day 14 of dosing were 2 to 3 times greater than those on Day 1 of dosing, and on Day 28 of dosing were similar to those on Day 14 of dosing. In males at the high dose level, mean plasma PROJECT Q concentration at 4 hours after dosing on Day 8 of dosing (final dosing at 600 mg/kg) was approximately 3 times greater than that on Day 1 of dosing (first dosing at 600 mg/kg). Mean plasma PROJECT Q concentration before dosing on Day 14 of dosing (approximately 24 hours after dosing at 300 mg/kg) was approximately half that on Day 9 of dosing (approximately 24 hours after the final dosing at 600 mg/kg). Mean Cmax and AUC24 on Day 14 of dosing were similar to those on Day 28 of dosing. Mean tmax were 1 to 7 hours at 20 to 600 mg/kg, and tended toward prolongation by dose escalation. There was no appreciable sex difference in any parameter.

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was 20 mg/kg/day for both sexes.