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

**11 SUMMARY**

PROJECT N suspended in the vehicle [0.5 w/v% methylcellulose (MC) solution] was orally administered to male and female beagle dogs at dose levels of 0.1, 1, and 10 mg/kg/day. The animals in the control group received 0.5 w/v% MC solution. Four males and 4 females were assigned to each group for toxicity evaluation during a 4-week treatment period. Additional 3 males and 3 females per group were assigned to the 1 and 10 mg/kg groups for reversibility evaluation during a subsequent 4-week recovery period. Systemic exposure to PROJECT N was also evaluated. The following observations and examinations were performed: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, blood pressure,

urinalysis, hematology, blood chemistry, analysis of bone and cartilage-related markers, gross pathology, organ weights, and histopathology.

At 0.1 mg/kg, decreased albumin, cartilage thickening in the rib, and atrophy with cellular infiltration in the tarsal gland were noted in both sexes. Increased alkaline phosphatase (ALP) and bone-ALP (BAP), and decreased 1,25-Dihydroxyvitamin D3 [1,25(OH)2D] were noted in males. In females, increased collagen type I and II cleavage (C1,2C) and decreased intact- parathyroid hormone (PTH) were noted.

At 1 mg/kg, soft stool, diarrhea, mucous stool, abnormal stool color (reddish, positive occult blood reaction), rough fur, loss of nails, decreased body weight and food consumption, positive urinary occult blood reaction, prolonged activated partial thromboplastin time (APTT), increased alanine transaminase, ALP, inorganic phosphorus (IP), C1,2C, chondroitin sulfate 846 (CS846), and BAP, decreased total protein, albumin, albumin/globulin ratio, triglycerides, total cholesterol, PTH, and 1,25(OH)2D, firm white focus in the aorta and heart, enlargement of costochondral junction in the rib, mineralization in the heart, aorta, and tongue, mononuclear cell infiltration and edema in the lung, epithelial atrophy in the tongue, esophagus, cornea of the eyeball, nail, and tarsal gland, cartilage thickening in the sternum, femur, and rib, and elongation of primary spongiosa in the rib were observed in both sexes. In males, decrease in spontaneous activity, decreased hematocrit value and reticulocyte ratio, increased globulin, edema in the pharyngeal muscle layer, hemorrhage in the lung, and atrophy of the mammary gland were observed. In females, increased urea nitrogen, brown focus in the tongue, firm and dark purple focus in the lung, and curvatura of the sternum were observed.

At 10 mg/kg, 1 male and 2 females died or were moribund euthanized on Day 16 or 22 of dosing. One male died on Day 8 of recovery due to deteriorated condition continuously noted from the dosing period. These animals showed similar findings to those noted in the surviving animals.

The following changes were noted in both the surviving and mortal animals. Soft stool, diarrhea, mucosal stool, abnormal stool color, decrease in spontaneous activity, prone or lateral position, tachypnea, dehydration signs, abnormal tongue color (dark), erosion of skin (decubitus), eye mucus, rough fur, loss of nails, decreased body weight and food consumption, increased heart rate, decreased sodium and chloride excretion in urine, increased erythrocyte count, hemoglobin concentration, hematocrit value, and large unstained cell count, decreased reticulocyte ratio, total leukocyte, lymphocyte, neutrophil, eosinophil, and basophil counts, prolonged APTT, increased aspartate transaminase, ALP, globulin, IP, CS846, and BAP, decreased albumin, albumin/globulin ratio, creatinine, calcium, sodium, chloride, and ionized calcium, red focus, dark brown discoloration, and loss of tip in the tongue, firm focus and/or white focus in the aorta, heart, lung, pharynx, kidney, skeletal muscle, spleen, and diaphragm, rough surface of the femoral diaphyses, enlargement of costochondral junction of the rib, firm and increased weight of the lung, small and decreased weight of the thymus, mineralization in the systemic organs, ulcer in the tongue, acinar atrophy in the sublingual gland, necrosis in the cortex of the kidney and skeletal muscle, hypocellularity in the sternal and costal bone marrow, erosion of the trachea, epithelial atrophy of the multiple organs (bronchus, tongue, esophagus, cornea, nail, mammary gland, and tarsal gland), atrophy of the thymus, Peyer’s patch, mononuclear cell infiltration in the lung, decreased glycogen in the hepatocytes, decreased zymogen granules in the pancreas, decreased lipid and hypertrophy of the zona fasciculata in the adrenal, cartilage thickening in the sternum, femur and rib, decreased trabecular bone/cortical bone in the sternum, increased extraperiosteal cartilage/bone in the femur, and elongation of the primary spongiosa in the rib were observed in both sexes. In males, suppression or disappearance of touch response, positive occult blood reaction in urine, erythrocytes in urinary sediment, increased neutrophil count, decreased triglycerides, urea nitrogen, firm and/or white focus in the stomach, colon, and subcutaneous, adhesion of the white material to the ilium, necrosis and mononuclear cell infiltration in the myocardium, thrombus in the kidney, ulcer in the skin, opacity, and erosion of the cornea and edema in the corneal stroma in the eyeball, and red discoloration and congestion in the mucosa of the duodenum and jejunum were observed. In females, salivation, hemorrhage in the tongue, increased total protein, triglycerides, total cholesterol, urea nitrogen, potassium, and PTH, decreased erythrocyte count, hemoglobin concentration, hematocrit value, platelet, monocyte, and large unstained cell counts, and total protein, brown focus in the tongue, dark red focus and hemorrhage in the mucosa of the jejunum, and edema and neutrophil infiltration in the lung were observed.

During the recovery period at 1 and 10 mg/kg, all changes observed during the dosing period recovered or tended to recover, except for mineralization in several organs and 1,25(OH)2D. Decreased erythrocyte count, hemoglobin concentration, and hematocrit value were noted in the animals at 10 mg/kg which did not show similar changes during the dosing period, and lens opacity was observed at 1 and 10 mg/kg in ophthalmology only at the examination during the recovery period.

In toxicokinetics, the mean Cmax and AUC24 values increased almost or nearly dose proportionally up to 1 mg/kg, and less than dose proportionally at 10 mg/kg. The Cmax and AUC24 values on Days 14 and 28 did not clearly differ from those on Day 1. There was no clear sex difference in any parameter.

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was less than 0.1 mg/kg/day for males and females since atrophy of the tarsal gland, changes in bone and cartilage-related markers, and cartilage thickening of the rib were noted at 0.1 mg/kg/day. All changes observed during the dosing period, except for mineralization at 1 mg/kg/day and greater, recovered or tended to recover at the end of the 4-week recovery period. Anemia and lens opacity were newly observed in the recovery period.