### Safety Pharmacology

A summary of safety pharmacology studies is presented in [[Table 7](#_bookmark27)].

When a single dose of PROJECT N was administered to male rats by gavage, the central nervous system (general condition and behavior) was not affected at doses up to 10 mg/kg (Study Project N-PT-0002).

When a single dose of PROJECT N was given to male dogs by gavage, vomiting was observed at doses 10 and 100 mg/kg and compound-colored feces, increased blood pressure (at 8 hours after dosing) and decreased heart rate (at 24 hours after dosing) were observed at 100 mg/kg; no effect was observed on the ECG (Study Project N-PT-0003).

At doses up to 10 µmol/L, PROJECT N did not inhibit the hERG current in HEK293 cells expressing hERG channels (Study Project N-PT-0001).

### Table 7 Overview of Safety Pharmacology

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Method** | **Species, System, Sex, Method of Administration** | **Items/Parameters Evaluated** | **Noteworthy Findings** | **Study Number** |
| hERG current | Whole cell patch clamp method | HEK293 cells expressing hERG channels  5 cells  0, 1, 3, 10 µmol/L | Inhibitory effect on hERG current | No effect | Project N-PT-0001 |
| Central nervous system | Modified Irwin’s method | Rats, SD, Male n = 6 per group  0, 1, 3, 10 mg/kg | General condition and behavior | None | Project N-PT-0002 |
| Central nervous system, cardiovascular system, respiratory system | Telemetry method | Dogs, Beagle, Male n = 4 per group  0, 1, 10, 100 mg/kg | General condition and behavior, body temperature, heart rate, blood pressure (SBP, DBP and mean BP), ECG (PR  interval, QT interval and QTc interval), respiration rate, blood gases and blood electrolyte concentrations | 1 mg/kg: No change  10 mg/kg: Vomiting  100 mg/kg: Vomiting, increased blood pressure, compound-colored feces and decreased heart rate | Project N-PT-0003 |

BP: blood pressure; DBP: diastolic blood pressure; ECG: electrocardiogram; HEK: human embryonic kidney; hERG: human ether-a-go-go-related gene; SBP: systolic blood pressure

### Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction studies with PROJECT N have been conducted.

## Toxicology

An overview of toxicology studies is presented in [End-of-Text Table 3.1].

### Single-dose Toxicity

A summary of the study is presented in [Table 9](#_bookmark51) and [End-of-Text Table 3.2].

A single dose of PROJECT N (suspended in 0.5 w/v% methylcellulose [MC] solution; 30, 100 and 300 mg/kg) was orally administered to 5 male and 5 female rats per dose group, and the acute toxicity of PROJECT N was evaluated. The observation period was 15 days starting from the day of administration to day 14.

In the 30 mg/kg group, no abnormality was observed in the general condition during the observation period. Suppression of body weight gain was observed in both sexes from day 1 (the day after administration) to 4. A decrease in food consumption was observed in

1 female from the day of administration to day 2. The necropsy findings showed white focus in the testis of 1 male, and histopathologically, seminiferous tubule atrophy was observed.

In the 100 mg/kg group, 1 male and 3 females died on day 4. No change in the general condition of rats that survived and of those that died was observed on the day of administration. However, a decrease in stool volume was observed on day 1. From day 2, reddish urine (positive for occult blood) was observed in both sexes, and reddish eye mucosa was observed in males. From day 3, a decrease in stool volume and mucus stool were observed in both sexes, and a decrease in spontaneous activity and abnormal respiratory tones were observed in males. On day 4, no stool was observed in males and emaciation was observed in females. In the rats that died on day 4, prone position, hypothermia, gasping and ataxic gait were also observed. In the rats that survived, no abnormality was observed in the general condition on day 8 and thereafter. A decrease in body weight, suppression of body weight gain and a decrease in food consumption were observed in males until day 7 and in females until day 4. The necropsy findings showed white focus in the heart, firm thoracic aorta, white focus in the stomach, firm stomach and firm colon. Corresponding to these gross lesions, mineralization was observed in histopathological examination. Other histopathological findings showed seminiferous tubule atrophy in all males that survived and glandular gastric mucosal hemorrhage in the rats that died.

In the 300 mg/kg group, all males and females either died or were sacrificed moribund between day 3 and day 4. On the day of administration, no change was observed in the general condition. A decrease in stool volume and mucus stool were observed in both sexes from day 1, and no stool was observed in females from day 2. From day 3, a decrease in spontaneous activity was observed in both sexes and no stool and reddish urine (positive for occult blood) in males. In the males sacrificed moribund on day 3, hypothermia and bradypnea were observed. On day 4, emaciation was observed in both sexes, and soiled perineal region was observed in females. A decrease in body weight and a decrease in food consumption were observed prior to death or moribund sacrifice. The histopathological findings did not show seminiferous tubule atrophy, which was observed in the 30 and

100 mg/kg groups. Mineralization in the stomach was observed only in 1 male. Hemorrhage was observed in the lung in 2 females, in the forestomach mucosa in 1 female, in the glandular stomach mucosa in 3 males and in the urinary bladder mucosa in 1 female.

The lethal dose was estimated to be 100 mg/kg in both sexes. The early acute symptoms were abnormal feces, including a decrease in stool volume, no stool and mucus stool.

Mineralization in multiple organs was observed as the noteworthy findings.

### Table 9 Overview of Single-dose Toxicity Study in Rats

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species, Strain, Method of Administration** | **Number of Animals**  **/Sex/ Group** | **Dose (mg/kg)** | **Lethal Dose** | **Noteworthy Findings** | **Study Number** |
| Rat, SD,  Gavage, Observed for 15 days | Male/ Female 5/5 | 30  100  300 | 100 mg/kg (Male/Female) | 30 mg/kg: Suppression of body weight gain, a decrease in food consumption and seminiferous tubule atrophy  100 mg/kg: Death (day 4: 1 male and 3 females), abnormal feces, a decrease in body weight, a decrease in food consumption, seminiferous tubule atrophy and mineralization in multiple organs | Project N-TX-  0001 |
|  |  |  |  | 300 mg/kg: Death/moribund sacrifice (day 3 and day 4: all male and females), abnormal feces, a decrease in body weight, a decrease in food consumption, mineralization in the stomach, and hemorrhage in the lung, stomach and urinary bladder |  |

### Repeat-dose Toxicity

Two preliminary repeat-dose studies were conducted in rats (Study Project N-TX-0008) and dogs (Study Project N-TX-0009) to select the doses to be used in the main good laboratory practice (GLP) study. A 13-week GLP oral repeated-dose toxicity study in

rats (Study Project N-TX-0012) and a 13-week GLP oral repeated-dose toxicity study in in dogs (Study Project N-TX-0013) are also discussed below.

### 4-Week Oral Repeated-dose Toxicity Study Followed by a 4-Week Recovery Study in Rats

PROJECT N, suspended in 0.5 w/v% MC, at 0, 0.3, 1 and 3 mg/kg was administered by gavage to 10 male and 10 female rats per dose group once daily for 4 weeks. After the dosing period, the 0, 1, and 3 mg/kg groups were observed for 4 weeks (recovery period) in order to evaluate the reversibility of the toxicity observed during the dosing period. In addition, at each dose level, a satellite group was added to evaluate the level of systemic exposure to PROJECT N. The following noteworthy findings were obtained.

In both sexes in the 0.3 mg/kg or more groups, an increase in the total excretion of calcium in urine, thickening of the cartilage in the rib and atrophy in the corneal epithelium were observed. In males, an increase in the serum concentration of tartrate-resistant acid phosphatase 5b (TRACP 5b) and a decrease in the serum concentration of

1,25-dihydroxyvitamin D3 (1,25[OH]2D) were observed. In females, an increase in the serum concentration of alkaline phosphatase (ALP) and inorganic phosphorous, a decrease in the total protein and albumin, a decrease in the albumin/globulin ratio, an increase in

bone-specific alkaline phosphatase (BAP), a decrease in parathyroid hormone (PTH), accumulation of foam cells in the lung, thickening of the cartilage in the vertebra, atrophy of the tarsal gland and tongue epithelium were observed. An increase in the lung weight was observed in females in the 0.3 and 1 mg/kg groups.

In both sexes in the 1 mg/kg or more groups, balling position and corneal opacity were observed in observation of the general condition, and histopathologically, mineralization of the cornea and stomach, thickening of the epiphyseal cartilage/articular cartilage of the femur bone, elongation of the primary spongiosa of the femur bone and rib and focal hemorrhage in the lung were observed. In males, an increase in the excretion of inorganic phosphorus in urine, an increase in the serum concentrations of ALP and BAP, a decrease in the serum concentration of PTH and accumulation of foam cells in the lung were observed. In females, a decrease in body weight and food consumption was observed; moreover, a decrease in urine pH and an increase in urinary protein and ketone bodies were observed in urinalysis; an increase in the serum AST, ALT, BUN, creatinine and TRACP 5b were observed in blood chemistry; curvature of the vertebra and sternum was observed in necropsy; and thickening of the cartilage of the sternum, bone decrease (sternum, femur and vertebra), epiphyseal necrosis of the hip joint, and atrophy of the mammary gland were observed in histopathological examination.

In the 3 mg/kg group, 1 female was sacrificed moribund on day 13, and 1 male died on

day 29 (day of scheduled necropsy). With regard to the females, because worsening of their general condition was prominent, dosing was terminated on day 14. During the following

4-week recovery period starting from day 15, 1 female died on day 2 of recovery and another on day 8 of recovery. Mineralization in the systemic organs may have partially contributed to the cause of death, but details were not clear. The findings observed in the rats that survived and those that died or were sacrificed moribund were comparable. In both sexes, a decrease in spontaneous activity, emaciation, a decrease in stool volume, soft stool and a decrease in the reticulocyte ratio were observed; moreover, mineralization (thoracic aorta, tongue, kidney and spinal meninx), epiphyseal necrosis of the femur bone, hypocellularity (sternal bone marrow, femoral bone marrow, vertebral bone marrow and costal bone marrow) and atrophy of lymphoid tissues (thymus, spleen, Peyer’s patch, submandibular lymph node and mesenteric lymph node) and subcutaneous adipose tissue were observed in histopathological examination. In males, a decrease in body weight/food consumption was observed; moreover, a decrease in urine pH, urine volume and total amount of excretion of electrolytes in urine and a positive result for occult blood in urine were observed in urinalysis; an increase in the serum concentration of AST, ALT, BUN, creatinine and inorganic phosphorus, and a decrease in the serum concentration of total protein, albumin and albumin/globulin ratio were observed in blood chemistry; curvatura of the vertebra and sternum, and enlargement of the caput on the rib were observed in necropsy; an increase in the lung weight was observed in organ weight measurement; and thickening of the cartilage (sternum and vertebra), bone decrease (sternum, femur and vertebra), epiphyseal necrosis of the hip joint and epithelial atrophy (tarsal gland, tongue and mammary gland) were observed in histopathological examination. In females, no stool, mucous stool and paralysis of the hind leg were observed; moreover, an increase in the erythrocyte count, hemoglobin and hematocrit value, a decrease or increase in the platelet count, and a prolongation of the prothrombin time and activated partial thromboplastin time (APTT) were observed in hematology; and an increase in the serum concentration of 1,25(OH)2D, mineralization (heart, duodenum and colon) and necrosis of the zona fasciculata in the adrenal were observed. These changes observed during the dosing period were reversible or partially reversible except for the changes associated with mineralization. The ophthalmologic examination conducted during the recovery period showed lens opacity in 1 male in the

3 mg/kg group. In addition, from day 26 of recovery, 1 female in the 1 mg/kg group

developed paralysis of the hind leg that had not been observed during the dosing period. Moreover, white discoloration of the incisor was observed in females in the 1 mg/kg group and males in the 3 mg/kg group, and histopathologically, atrophy/necrosis of ameloblasts and disappearance of pigmented enamel were observed.

The level of systemic exposure was evaluated in the satellite group. The results showed that both Cmax and AUC24 increased in a dose-dependent manner. A difference in the dosing duration had no effect on Cmax and AUC24. In all dose groups, the level of exposure was higher in females than in males, and the AUC24 of the females was 1.5 to 2.0 times greater than that of the males.

These data suggest that NOAEL is less than 0.3 mg/kg in both sexes. Considering that death occurred in the 3 mg/kg group and that no serious toxicity was observed in the 1 mg/kg group, the STD10 was estimated to be 1 mg/kg.

### 13-Week Oral Repeated-dose Toxicity Study Followed by a 13-Week Recovery Study in Rats

Detailed information on this study is presented in [End-of-Text Table 3.2.2].

PROJECT N, suspended in 0.5 w/v% MC, at 0, 0.1, 0.3 and 0.6 mg/kg was administered by gavage to 10 male and 10 female rats per dose group once daily for 13 weeks. Five males and 5 females were added to the control and 0.6 mg/kg groups in order to assess the reversibility of toxicity observed during the dosing period in a subsequent 13-week recovery period. In addition, at each dose level, a satellite group was added to evaluate the level of systemic exposure to PROJECT N. For females at 0.6 mg/kg, the dosing period was reduced to 65 days (toxicity group) or 66 days (satellite group) due to deteriorated general condition and marked body weight loss, followed by a 13-week recovery period.

At 0.1 mg/kg and above, thickening of the cartilage in the digit bone was observed in both sexes; increased serum TRACP 5b was observed in males; and increased urinary total inorganic phosphorus excretion, increased serum BAP, and hyperkeratosis of the nail and atrophy of the corneal epithelium was observed in females .

At 0.3 mg/kg and above, elongation of the nails was observed in both sexes. In males, increased urinary total inorganic phosphorus excretion, decreased serum intact PTH, and hyperkeratosis of the nail and atrophy of the corneal epithelium were observed. In females, the following changes were observed: balling position and trace of reddish rhinorrhea; decreased body weight; decreased urinary pH and increased urinary total calcium excretion; increased leukocyte counts (increased neutrophil, lymphocyte, and monocyte counts); increased serum creatinine and gamma-globulin ratio, and decreased serum albumin concentration and ratio and albumin/globulin ratio; increased serum TRACP 5b (only

0.3 mg/kg); curvatura in the vertebra, enlargement of the caput in the rib, and foam cell accumulation in the lung with gross white focus and increased organ weight; thickening of the cartilage in the sternum, rib, and vertebra, and atrophy of the tarsal gland and tongue epithelium; and incisor changes (gross whitening, defect, and/or elongation of the incisor, and atrophy and necrosis of the ameloblasts, disappearance of the pigmented enamel, and inflammation in the gingiva).

At 0.6 mg/kg, females showed decreased body weight gain from Week 4 of dosing. From Week 8 of dosing, marked body weight loss was noted. On day 65 of dosing, 1 female showed decreased spontaneous activity and was then found dead at approximately 3 hours after dosing. Because general conditions of other females were similar to those of the animal that died, 9 females were necropsied on day 66 of dosing, and the remaining 5 females were used to assess the reversibility of toxicity during the 13-week recovery period. It was considered that marked body weight loss and deteriorated general condition contributed to

the cause of death. Pathological findings in the animal that died were almost the same as those in the animals that survived at 0.6 mg/kg.

In both sexes, the following changes were observed: corneal opacity, anterior corneal deposits (ophthalmology examination), and mineralization in the cornea; decreased food consumption; decreased serum glucose; erosion in the glandular stomach (gross black focus in the mucosa); and thickening of the epiphyseal cartilage and articular cartilage in the femur and mineralization in the renal cortex.

In males, the following changes were observed: balling position and trace of reddish rhinorrhea; decreased body weight; increased urinary calcium excretion; increased neutrophil count; increased serum creatinine; curvatura in the vertebra and foam cell accumulation in the lung with gross white focus; thickening of the cartilage in the sternum, rib, and vertebra, and atrophy of the tarsal gland and tongue epithelium; and incisor changes (gross whitening, defect, and/or elongation of the incisor, and atrophy and necrosis of the ameloblasts, disappearance of the pigmented enamel, and inflammation in the gingiva).

In females, the following changes were observed: decreased spontaneous activity, emaciation, soiled perineal region, and decreased stool volume; decreased urinary total sodium, potassium, and chloride excretions; decreased reticulocyte ratio; and increased serum inorganic phosphorus, increased β-globulin ratio and decreased serum total protein and calcium; decreased serum intact-PTH; curvatura in the sternum, rough surface of the caput in the femur, thickening of the rib, adhesion of white material to the hip joint, atrophy of thymus and spleen with decreased organ weights, and dilatation of the cecum; and elongation of the primary spongiosa and epiphyseal necrosis in the femur, decreased trabecular bone and cortical bone in the sternum and vertebra, mineralization in the meninx in the spinal cord and glandular mucosa of the stomach, cellular infiltration of the subepithelium and erosion in the tongue, necrosis of the dental pulp and odontoblasts, hypocellularity in the sternal bone marrow, femoral bone marrow, costal bone marrow, and vertebral bone marrow, atrophy of the mammary gland, mesenteric lymph nodes, Peyer’s patches and adipose tissue in the skin, and decreased zymogen granules in the pancreas.

During the 13-week recovery period, the following changes were observed: epiphyseal necrosis in the femur with increased neutrophil count, thickening of the cartilage in the digit bone, decreased intact-PTH, incisor changes (gross whitening, defect, and/or elongation of the incisor, and atrophy and necrosis of the ameloblasts and disappearance of the pigmented enamel, inflammation in the gingiva, and necrosis of the odontoblasts), and secondary changes related to the incisor damage such as decreased food consumption with decreased body weight, decreased urinary pH, urinary total electrolyte (sodium, potassium, and chloride) excretions, and changes in serum protein. Bone and cartilage-related changes except for those described above, mineralization-related changes, and lung changes were still observed at the end of the recovery period, but they showed tendency toward recovery. The other changes observed during the dosing period disappeared by the end of the recovery period. Dragging of the hindlimbs was newly observed in only 1 female from day 84 of

recovery and the fracture of the intervertebral disk in the vertebra was revealed; therefore, this change might occur during bone remodeling for recovery.

In toxicokinetics, Cmax and AUC24 in both sexes increased dose-proportionally during the dosing period. Cmax and AUC24 at 0.1 and 0.3 mg/kg were slightly higher on day 56 of dosing than on day 1 of dosing, but there was no remarkable difference between days 56 and 91 of dosing. Cmax and AUC24 at 0.6 mg/kg were almost the same levels during the dosing period. AUC24 was 1.5 to 2.1 fold higher in females than in males.

As bone and cartilage-related changes in both sexes and atrophy of the corneal epithelium in females were observed at 0.1 mg/kg, it was concluded that under the conditions of this study, the NOAEL for males and females was lower than 0.1 mg/kg. The changes observed at

0.6 mg/kg during the dosing period recovered or tended to recover during the 13-week recovery period, except for incisor-related changes epiphyseal necrosis in the femur, and cartilage thickening of the digit bone, and decreased intact-PTH, and increased neutrophil count in both sexes.

### 4-Week Oral Repeated-dose Toxicity Study Followed by a 4-Week Recovery Study in Dogs

PROJECT N, suspended in 0.5 w/v% MC, at 0, 0.1, 1 and 10 mg/kg was repeatedly administered by gavage to 4 male and 4 female beagle dogs per dose group once daily for 4 weeks. After the dosing period, the 1 and 10 mg/kg groups were observed for 4 weeks (recovery period) in order to evaluate the reversibility of the toxicity observed during the dosing period. In addition, the level of systemic exposure to PROJECT N was evaluated at each dose level. The following noteworthy findings were obtained.

In both sexes in the 0.1 mg/kg or more groups, a decrease in the serum concentration of albumin, thickening of the cartilage in the rib and atrophy of the tarsal gland were observed. In males, an increase in the serum concentration of ALP and BAP was observed. In the 0.1 and 1 mg/kg groups, a decrease in the concentration of 1,25(OH)2D was observed in males, and an increase in the concentration of collagen type I and II cleavage (C1,2C) and a decrease in the concentration of PTH were observed in females.

The following changes were observed in the 1 mg/kg or more groups. In observation of the general condition, soft stool, diarrhea, mucous stool, reddish stool color, rough fur and loss of nails were observed in both sexes. Moreover, a decrease in body weight/food consumption and a positive result for occult blood (in females, this was observed only in the 1 mg/kg group) were observed. In hematology, a prolongation of APTT was observed. In blood chemistry, an increase in ALT, inorganic phosphorous and chondroitin sulfate 846 (CS846), and a decrease in the albumin/globulin ratio were observed. In necropsy, white focus in the thoracic aorta and heart, and enlargement of the costochondral junction of the rib were observed. In histopathological examination, mineralization (heart, thoracic aorta and tongue), mononuclear cell infiltration and edema in the lung, epithelial atrophy (tongue, esophagus, cornea and nail), thickening of the cartilage (sternum and femur bone), and elongation of the primary spongiosa of the rib were observed in both sexes. In males, a

decrease in the reticulocyte ratio, an increase in the concentration of C1,2C and a decrease in the concentration of PTH were also observed; histopathologically, hemorrhage in the lung (only in the 1 mg/kg group) and atrophy of the mammary gland were observed. In females, an increase in the concentration of BUN, ALP and BAP was observed; while in necropsy, curvature of the sternum (only in the 1 mg/kg group) was observed.

In the 10 mg/kg group, 1 male on day 16 and 2 females on day 22 died or were sacrificed moribund. In addition, 1 male died on day 8 of recovery due to unresolved worsening of the general condition that was first observed during the dosing period. An increase in heart rate was observed in ECG; a decrease in the excretion of sodium and chloride in urine, a decrease in the leukocyte count, lymphocyte count, neutrophil count, eosinophil count and basophil count, an increase in the concentration of AST, a decrease in the serum concentration of calcium and ionized calcium, color abnormality of the tongue, loss of the tip of the tongue, systemic mineralization, hypocellularity in the costal bone marrow and sternal bone marrow, epithelial atrophy of the bronchus, and an increase in the femoral extraperiosteal cartilage/bone were also observed in both sexes. In males, the appearance of erythrocytes in urinary sediment was also observed. In females, a decrease in the reticulocyte ratio, platelet count, monocyte count and large unstained cell count, and atrophy of the mammary gland were observed.

After the 4-week recovery period, all changes observed during the dosing period were fully or partially reversible except for the mineralization and the changes associated with mineralization. The changes that appeared after the recovery period were lens opacity (both sexes in the 1 mg/kg group, and females in the 10 mg/kg group), and a decrease in the erythrocyte count, hemoglobin and hematocrit value (both sexes in the 10 mg/kg group).

The level of systemic exposure was evaluated. The results showed that both Cmax and AUC24 increased in an almost dose-dependent manner at doses up to 1 mg/kg. However, at

10 mg/kg, the Cmax and AUC24 were lower than the values expected from the proportion. The duration of dosing had no effect on the level of systemic exposure. No difference between the sexes was observed.

These data suggest that NOAEL is less than 0.1 mg/kg in both sexes. Considering that a serious toxicity (mineralization of the heart and thoracic aorta) was observed in the 1 mg/kg group and that no serious toxicity was observed in the 0.1 mg/kg group, the HNSTD was estimated to be 0.1 mg/kg.

### 13-Week Oral Repeated-dose Toxicity Study Followed by a 13-Week Recovery Study in Dogs

An overview of this study is presented in [End-of-Text Table 3.2.4].

PROJECT N, suspended in 0.5 w/v% MC, at 0, 0.03, 0.1 and 0.3 mg/kg was repeatedly administered by gavage to 4 male and 4 female beagle dogs per dose group once daily for

13 weeks. It was decided to terminate dosing to males in the 0.3 mg/kg group on day 72 due to difficulty in standing. After the dosing period, the 0.3 mg/kg group was observed for

13 weeks (recovery period) in order to evaluate the reversibility of the toxicity observed during the dosing period.

At 0.03 mg/kg, atrophy with cellular infiltration in the tarsal gland and thickening of the epiphyseal cartilage in the femur were noted in both sexes. Atrophy of the corneal epithelium and enlargement of the costochondral junction and thickening of the cartilage in the rib were noted in males.

At 0.1 mg/kg, loss of nails, decreased albumin and albumin/globulin ratio, enlargement of costochondral junction in the rib, elongation of fur (eyelash, digital extremes, and/or whole body), atrophy with cellular infiltration in the tarsal gland, atrophy of the corneal epithelium, thickening of the cartilage in the sternum and rib, thickening of the epiphyseal cartilage in the femur, and atrophy/necrosis of the epithelium and inflammation in the nail bed were noted in both sexes. In males, rough fur, eye mucus, focal corneal opacity, posterior capsular lens opacity and subcapsular degeneration of the lens fiber at the posterior pole, and decreased PTH and 1,25(OH)2D were noted. In females, increased CS846 was noted.

At 0.3 mg/kg, 3 males showed difficulty in standing and it was decided to terminate dosing to males on day 72. In these 3 males, moderate necrosis of the synovial tissue and the articular cartilage in the femur in histopathology were revealed and considered to be the cause of difficulty in standing. The changes noted at 0.3 mg/kg including these males were as follows: rough fur, loss of nails, abnormal gait (no attempt to stand up and/or walk, not standing on all limbs, hunched back and/or straightened hind limbs), eye mucus, decreased body weight, decreased food consumption, focal or diffuse corneal opacity, increased systolic and diastolic pressure, increased CS846, decreased PTH and 1,25(OH)2D, increased ALT, decreased albumin and albumin/globulin ratio, enlargement of the costochondral junction in the rib, firm tissue or white focus with firm tissue in the lungs, elongation of fur (eyelash, digital extremes, and/or whole body), increased lung weight, atrophy with cellular infiltration in the tarsal gland, atrophy of the corneal epithelium, atrophy of the epithelium in the tongue and esophagus, thickening of the cartilage in the sternum and rib, elongation of the primary spongiosa in the rib, thickening of the epiphyseal cartilage in the femur, and atrophy/necrosis of the epithelium and inflammation in the nail bed, mineralization in the bronchiole and alveolar septa, mononuclear cell infiltration, edema, and bronchiolo-alveolar hyperplasia in the lungs in both sexes; reddish soft stool or reddish mucous stool which were positive for occult blood reaction, prolongation of APTT, increased ALP, globulin, and inorganic phosphorus, decreased triglycerides, deformity of the caput with adhesion of white material, and thickening of the distal end in the femur, necrosis of the epiphyseal cartilage, hyperplasia and necrosis of the synovial tissue, thickening and necrosis of the articular cartilage in the femur, and hemorrhage in the lungs in males; corneal pannus, and posterior capsular lens opacity and subcapsular degeneration of the lens fiber at the posterior pole in females.

At the end of the 13-week recovery period, rough fur remained, including newly observed in 1 male during the recovery period, but it recovered in 1 female and other animals showed slight recovery. The posterior capsular lens opacity did not recover and was newly observed in 2 males during the recovery period. Findings which were observed during dosing period

did not progress during the recovery period. The corneal opacity which was observed during

dosing period did not recover during the recovery period and degeneration/necrosis of the corneal epithelium was observed at the end of recovery period. Curvatura of the sternum was observed in 1 female without necrosis or thickening of the cartilage. Disarrangement of the cartilage in the sternum and rib were observed in males and females, and these findings were considered to be remodeling process of the thickening of the cartilage at the end of dosing period. Decreased PTH that remained in males and females was thought to be related to these bone and cartilage changes. Recovery was shown for the other changes by the end of the recovery period.

During the dosing period, tmax values ranged from 0.5 to 1.6 h. Mean Cmax and AUC24 values increased almost dose proportionally in both sexes on days 1, 56, and 91 of dosing. No parameters were influenced by repeated dosing. Mean Cmax and AUC24 values in males at

0.3 mg/kg on days 28 and 72 were similar to those on days 1 and 56. There were no clear sex differences in tmax, Cmax, or AUC24 at any dose.

It was concluded that, under the conditions of this study, the NOAEL was less than

0.03 mg/kg per day for males and females, since atrophy of the tarsal gland and thickening of the femoral epiphyseal cartilage were noted in both sexes and corneal atrophy and thickening of the rib cartilage were noted in males at 0.03 mg/kg per day. All test article-related changes were reversible, tended toward reversibility, or were not progressive during the

13-week recovery period.

### Toxicokinetics

[Table 10](#_bookmark53) summarizes the toxicokinetic parameters evaluated in the oral repeat-dose toxicity studies of PROJECT N in rats and dogs. In the 13-week oral repeat-dose toxicity study in rats, both Cmax and AUC24 increased in a dose-dependent manner, as in the 4-week oral

repeat-dose toxicity study. The duration of dosing had no effect on these parameters. In all dose groups, the level of exposure was higher in female rats than that in male rats. The AUC24 in female rats was 1.5 to 2.0 times greater in the 4-week oral repeat-dose toxicity study, 1.6 to 1.8 times in the 13-week repeat-dose toxicity study.

In the 4-week oral repeat-dose toxicity study in dogs, both Cmax and AUC24 increased in an almost dose-dependent manner at doses up to 1 mg/kg. However, at 10 mg/kg, the Cmax and AUC24 were lower than the values expected from the proportion. In the 13-week oral repeat-dose toxicity study, both Cmax and AUC24 increased in an almost dose-dependent manner. The duration of dosing had no effect on these parameters. No difference was observed between both sexes.

PROJECT N CONFIDENTIAL

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### Table 10 Toxicokinetic Parameters of PROJECT N after Oral Administration of PROJECT N in Rats and Dogs

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Number of Animals** | **Sex** | **Dose (mg/kg)** | **Unchanged Drug Cmax (ng/mL)** | | | | **Unchanged Drug AUC24 (ng·h/mL)** | | | |
| **4-week study** | | **13-week study** | | **4-week study** | | **13-week study** | |
| **First Dose** | **Last Dose** | **First Dose** | **Last Dose** | **First Dose** | **Last Dose** | **First Dose** | **Last Dose** |
| Rat | 3 | Male | 0.1 | NA | NA | 29.6 | 67.8 | NA | NA | 183 | 268 |
| 3 | Female | 0.1 | NA | NA | 55.9 | 94.6 | NA | NA | 352 | 471 |
| 3 | Male | 0.3 | 98.7 | 149.8 | 111 | 210 | 454.0 | 634.3 | 558 | 1030 |
| 3 | Female | 0.3 | 166.1 | 230.0 | 153 | 295 | 897.1 | 1133.3 | 979 | 1730 |
| 3 | Male | 0.6 | NA | NA | 202 | 278 | NA | NA | 1210 | 1490 |
| 3 | female | 0.6 | NA | NA | 305 | 223‡ | NA | NA | 2120 | 2420 ‡ |
| 3 | Male | 1 | 347.7 | 451.6 | NA | NA | 1592.6 | 2075.0 | NA | NA |
| 3 | Female | 1 | 575.3 | 508.1 | NA | NA | 3261.4 | 3330.9 | NA | NA |
| 3 | Male | 3 | 1105.2 | 915.0 | NA | NA | 6310.4 | 5277.5 | NA | NA |
| 3 | Female | 3 | 1387.5 | 1553.3† | NA | NA | 9538.4 | 11468.7 † | NA | NA |
| Dog | 4 | Male | 0.03 | NA | NA | 21.4 | 25.4 | NA | NA | 106 | 154 |
| 4 | Female | 0.03 | NA | NA | 22.7 | 28.5 | NA | NA | 141 | 181 |
| 4 | Male | 0.1 | 84.3 | 56.5 | 65.0 | 73.3 | 456.4 | 349.4 | 414 | 519 |
| 4 | Female | 0.1 | 88.0 | 71.4 | 66.7 | 84.4 | 523.6 | 414.6 | 374 | 481 |
| 7 | Male | 0.3 | NA | NA | 177 | 164 § | NA | NA | 1100 | 1370 § |
| 7 | Female | 0.3 | NA | NA | 194 | 178 | NA | NA | 1300 | 1300 |
| 7 | Male | 1 | 699.5 | 445.9 | NA | NA | 4644.1 | 2815.2 | NA | NA |
| 7 | Female | 1 | 455.2 | 614.1 | NA | NA | 3496.5 | 4073.8 | NA | NA |
| 7 | Male | 10 | 2135.0 | 1429.5 (n = 6) | NA | NA | 19738.4 | 11310.9 (n = 6) | NA | NA |
| 7 | Female | 10 | 1935.6 | 2383.4 (n = 5) | NA | NA | 14799.4 | 15687.1 (n = 5) | NA | NA |

NA: not applicable

†Values on day 14

‡Values on day 66

§Values on day72

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### Genotoxicity

An overview of the studies is presented in [Table 11.](#_bookmark55)

A reverse mutation study was conducted using *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and *E. coli* (WP2*uvrA*). In all bacterial strains tested, the revertant colony counts did not increase at doses up to 250 µg/plate without metabolic activation and up to

500 µg/plate with metabolic activation. Thus, PROJECT N did not induce reverse mutation (Study Project N-TX-0004).

A chromosomal aberration study was conducted using Chinese hamster lung (CHL/IU) cells. A significant increase in the number of cells with numerical chromosomal aberrations was observed at 109 µg/mL after short-term treatment (6 h) without metabolic activation, and at 0.723 and 0.868 µg/mL after long-term treatment (24 h) without metabolic activation, as well as at 109 and 153 µg/mL after short-term treatment (6 h) with metabolic activation

(Study Project N-TX-0005).

In the mouse micronucleus study (PROJECT N at 7.5, 15 and 30 mg/kg) was orally administered to male ICR mice for 2 days), an increase was observed in the percent ratio of polychromatic erythrocytes with micronuclei in femoral bone marrow cells in the 30 mg/kg group.

PROJECT N induced micronuclei (Study Project N-TX-0006).

Although a negative result was obtained for the reverse mutation study, the result of the in vitro chromosome aberration study was positive, and PROJECT N also induced micronuclei in the mouse micronucleus study. Thus, PROJECT N was found to be genotoxic.

### Table 11 Outline of Genotoxicity Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Species Strains, and**  **Methods of Administration** | **Number of Animals/ Sex/**  **Group** | **Concentration** | **Noteworthy Findings** | **Study Number** |
| Reverse mutation | *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and *E. coli*  (WP2*uvrA*) | − | Without S9: 7.81 to 250 µg/plate  With S9:  15.6 to 500 µg/plate | − | Project N-TX-  0004 |
| Chromosome aberrations | CHL/IU cells  6 h treatment or 24 h treatment with or without metabolic activation (S9) | − | 6 h treatment Without S9: 13.7 to 109 µg/mL  With S9:  13.7 to 153 µg/mL 24 h treatment Without S9:  0.181 to 0.868 µg/mL | 6-hour treatment Without S9: 109 µg/mL With S9:  109 and 153 µg/mL  24 h treatment Without S9: A significant increase in the number of  cells with numerical chromosomal aberrations was observed at  0.723 and 0.868 µg/mL | Project N-TX-  0005 |
| Micronuclei | Mouse, ICR, 2- day administration by  gavage | Male n = 5 | 0, 7.5, 15, 30 mg/kg | 7.5 and 15 mg/kg: Negative 30 mg/kg: A significant  increase in the percent ratio of PCEs with micronuclei was observed in femoral bone marrow cells | Project N-TX-  0006 |

-: no noteworthy findings were observed; PCE: polychromatic erythrocyte

### Carcinogenicity

The proposed indication for PROJECT N is solid tumor. Thus, on the basis of the ICH-S9 Guideline, no carcinogenicity study has been planned.

### Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies have been conducted.

### Local Tolerance

No local tolerance study was conducted.

### Other Toxicity Studies

As other toxicity studies, a phototoxicity study (GLP) using cultured mammalian cells and a 4-week oral repeated-dose study of PROJECT N (Non-GLP) in rats fed low-phosphate diets were conducted.

### Phototoxicity

In the in vitro phototoxicity study using cultured mammalian cells (Balb/c 3T3 cells), PROJECT N did not induce phototoxicity (Study Project N-TX-0007).

### 4-Week Oral Repeated-dose Study of PROJECT N in Rats Fed Low-phosphate Diets

An outline of Studies Project N-TX-0011 and Project N-TX-0002 is presented in [[Table 12](#_bookmark62)].

Study Project N-TX-0011 was conducted to determine whether PROJECT N-induced mineralization can be mitigated by decreasing the absorption of phosphorus from the intestinal tract with low-phosphate diets (containing a reduced concentration of phosphorus than normal feed). In the low-phosphate group, 5 male rats were maintained on low-phosphate diets (phosphorus concentration: 0.22%) and orally given 0 (vehicle control group: 0.5 w/v% MC), 0.3, 1 and

3 mg/kg of PROJECT N for 4 weeks. In the normal diet group, rats were maintained on a normal feed (phosphorus concentration: 0.81%) and orally given 0 (vehicle control group: 0.5 w/v% MC), 1 and 3 mg/kg of PROJECT N for 4 weeks. In addition, satellite groups (only PROJECT N- treated groups: 2 males per group) were used to evaluate the systemic exposure to PROJECT N at each dose level.

One rat each from the normal diet and low-phosphate diet groups given 3 mg/kg of PROJECT N were sacrificed moribund. In these groups, abnormal feces, balling position, low body weight and a decrease in food consumption were observed, although no obvious difference was observed in the incidence and severity between the groups. The serum concentration of inorganic phosphorus was slightly higher in the low-phosphate diet group than in the normal diet group. This was suspected to be the effect of overnight fasting from 17 o’clock on the day before necropsy (day 28). Thus, the concentration of inorganic phosphorus was measured in the preserved plasma obtained from the satellite groups (without fasting). In the satellite groups, the concentration of inorganic phosphorus was slightly lower in the

low-phosphate diet group than in the normal diet group. The histopathological examination showed that, in the normal diet and low-phosphate diet groups given 3 mg/kg, mineralization

of the heart, thoracic aorta, stomach, kidney, and cornea were observed, although when compared the incidence of the mineralization in the stomach that was frequently observed with a high severity in the 4-week oral repeated-dose toxicity study followed by a 4-week recovery study in rats it was lower in the low-phosphate diet group than in the normal diet group (1 rat in the low-phosphate diet group and 5 rats in the normal diet group). With regard to the systemic exposure, the Cmax and AUC24 were slightly higher in the

low-phosphate diet group than in the normal diet group.

These data suggest that PROJECT N-induced mineralization can be mitigated by decreasing the absorption of phosphorus from the intestinal tract.

Moreover, because histopathological examination of the teeth was not conducted at the time of completion of dosing in the 4-week oral repeated-dose toxicity study followed by a

4-week recovery study in rats (Study Project N-TX-0002), pathological examination of the incisor and molar was conducted at the time of completion of the 4-week dosing with PROJECT N in this study, and histopathological changes in teeth were evaluated. The results showed that, in both the normal diet and low-phosphate diet groups, degeneration/necrosis of odontoblasts and irregular dentin formation in the incisor were observed in the 1 mg/kg or more groups, and degeneration/necrosis of ameloblasts was observed in the 3 mg/kg group. No abnormal change was observed in the molar.

### Table 12 Outline of 4-Week Low-phosphate Diet Study in Rats

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phosphorus Concentration in Feed (%)** | **0.81**  **(Normal Diet Group)** | | | **0.22**  **(Low-phosphate Diet Group)** | | | |
| **PROJECT N** | **0** | **1** | **3** | **0** | **0.3** | **1** | **3** |
| General condition | − | − | Death (1 male), decrease in spontaneous activity, balling position, abnormal feces, hypothermia, salivation, reddish rhinorrhea, soiled perineal region, emaciation and corneal opacity | − | − | − | Death (1 male), decrease in spontaneous activity, balling position, abnormal feces, bradypnea, salivation, reddish rhinorrhea, soiled perineal region and emaciation |
| Body weight/Food consumption | − | − | Low body weight, suppression of body weight gain and decrease in food consumption | − | − | − | Low body weight, suppression of body weight gain and decrease in food consumption |
| Serum concentration of inorganic phosphorus (mg/dL)  † | 8.6 | 9.2 | 10.0 | 9.0 | 10.0 | 9.9 | 12.6 |
| Plasma concentration of inorganic phosphorus (mg/dL) ‡ | NA | 7.4 | 8.1 | NA | 5.5 | 5.3 | 4.6 |
| Mineralization | − | − | +  Mineralization in the stomach 5/5 | − | − | − | +  Mineralization in the stomach 1/5 |
| Cmax (ng/mL) on day 28 of dosing with PROJECT N | NA | 453.7 | 773.7 | NA | 248.2 | 854.1 | 1541.5 |
| AUC24 (ng·h/mL) on day 28 of dosing with PROJECT N | NA | 2098.6 | 4954.0 | NA | 1032.0 | 2810.3 | 4932.8 |

+: Present, −: Absent, NA: Not applicable

†: The serum concentration measured at the time of scheduled necropsy. The animals were fasted from 5:00 pm on the day before necropsy.

‡: The plasma concentration measured at 24 hours after dosing on day 28 in the satellite groups. The animals were not fasted.

## 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

In the 4-week oral repeat-dose toxicity studies in rats and dogs, death or moribund sacrifice occurred at 3 mg/kg in rats and at 10 mg/kg in dogs. In the 13-week oral repeat-dose toxicity studies, death and moribund sacrifice occurred at 0.6 mg/kg in rats, but did not occur in dogs. Prior to death or moribund sacrifice, worsening of the general condition such as body weight loss and decreased food consumption were observed.

Mineralization in multiple organs was observed in the 4 and 13-week studies in rats and dogs, and almost recovered after the 13-week recovery period. These data indicate that

mineralization needs long time to recover; therefore, it is important to prevent occurrence of mineralization in the clinical studies of PROJECT N. In the 4 and 13-week studies in rats and dogs, increased serum phosphorus was observed at lower or the same dose levels at which mineralization was observed. PTH and 1,25(OH)2D related to the metabolism of serum phosphorus and calcium decreased in rats and dogs in the 4 and 13-week studies at lower dose level in which no mineralization was observed. These data suggest that the serum phosphorus, PTH, and 1,25(OH)2D can be used as useful biomarkers [[Table 13](#_bookmark64)] to prevent mineralization. When PROJECT N was orally administered to rats fed low-phosphate diets for 4 weeks, the incidence of mineralization was lower in the low-phosphate diet group than in the normal diet group.

Abnormal feces such as soft stool and mucous stool were observed in both rats and dogs in the 4-week studies, and in dogs in the 13-week study. These changes rapidly disappeared after cessation of dosing. Hemorrhage in the jejunal mucosa in a dog at 10 mg/kg was the only relevant histopathological finding with abnormal feces. Subjective symptoms should carefully be monitored.

Reversible epithelial atrophy was observed in many organs and tissues such as the cornea, mammary gland, tongue, and tarsal grand at the lowest dose and greater in the 4 and 13-week studies in rats and dogs. In contrast to the atrophy, hyperkeratosis of the nail in rats and elongation of fur in dogs were also observed in the 13-week studies. Subjective symptoms such as hair loss, eye pain, skin disorder, nail disorder and stomatitis should be continuously observed.

Thickening of the cartilage was observed at the lowest dose and greater in the 4 and 13-week studies in rats and dogs and disappeared or mitigated after recovery period. The change occurred in the epiphyseal and articular cartilages of the femur, and cartilages of the rib, sternum and vertebra. The marked thickening of cartilage in the femur (hip joint) was developed to necrosis in dogs in the 13-week study as well as in rats in the 4-week study; however, dogs showed more severe condition (difficulty in standing) than rats. Subjective symptoms such as joint pain and joint deformity should to be continuously observed.

Decreases in trabecular and cortical bone were observed at high doses in the 4 and 13-week studies in rats and the 4-week study in dogs. The pathogenesis might be used as the source of calcium in response to increased serum phosphorus. These changes in cartilage and bone occurred with accompanying increases in serum CS846 (cartilage synthesis marker) and C1, 2C (cartilage degradation marker) in dogs, increases in serum TRACP 5b (osteoclast marker) and BAP (osteoblast marker) in rats. These biomarkers might be useful to detect the effect of PROJECT N on cartilage and bone [[Table 14](#_bookmark65)]. In addition, the use of diagnostic imaging such as simple X-ray and CT scans might be useful as well.

In the 4 and 13-week study in rats, foam cell accumulation in the lung was observed at the same dose level and recovered. In the lung of dogs, mononuclear cell infiltration, edema and hemorrhage were observed in the 4 and 13-week studies, and bronchiolo-alveolar hyperplasia was also observed in the 13-week study. These changes in dogs almost recovered, except for moderate mononuclear cell infiltration associated with the severe mineralization in the

4-week study. It was considered that these changes in the lung can be effectively diagnosed by symptom observation and the use of diagnostic imaging such as simple X-ray and CT scans.

In the 4-week studies, lens opacity was observed in rats and dogs during the recovery period. In the 13-week study in dogs, lens opacity corresponding to histopathological degeneration of lens fiber was observed during the dosing period, and not recovered with 13-week withdrawal. Considering that atrophy of the corneal epithelium was also observed in both rats and dogs, ophthalmologic examination is considered necessary in the clinical studies.

Paralysis of the hind leg was sporadically observed not only during the dosing period but also during the recovery period; therefore, the reversibility of this change was uncertain in the

4-week study in rats. In the animals with paralysis during the dosing period and the recovery period, balling position associated with curvature of the vertebra caused by cartilage thickening was observed at the same time. Therefore, an abnormal position of the vertebra might cause a peripheral nerve disorder and induce paralysis. No similar peripheral nerve disorder was observed in dogs. Observation of symptoms, the use of diagnostic imaging such as simple X-ray and CT scans, or electrophysiological tests are considered helpful in determining the cause of peripheral nerve disorder.

In the 4 and 13-week studies in rats and the 4-week study in dogs, decreased reticulocyte ratio and hypocellularity of the bone marrow were observed and recovered. Moreover, in the 4-week study in dogs, decreases in the erythrocyte count, hemoglobin and hematocrit value were observed during the recovery period. Thus, hematology testing is considered necessary in the clinical studies.

Increases in ALT and AST were observed in the 4-and 13-week studies in rats and dogs, and increases in creatinine and/or BUN were observed in the 4-week studies in rats and dogs and the 13-week study in rats. These changes were accompanied with no histopathological changes in the liver or kidney, and they were reversible after the 4 and 13-week recovery periods. Therefore, they may not indicate high potential risk of renal or hepatic toxicity in human, however, blood chemistry testing is considered necessary in the clinical studies because the mechanism was not clear.

In the single-dose telemetry study in dogs, mildly decreased heart rate and increased blood pressure were observed at 100 mg/kg of the highest dose. Reversible increased heart rate at 10 mg/kg and increased blood pressure at 0.3 mg/kg were observed in the 4 and 13-week studies in dogs, respectively. The measurement of blood pressure and heart rate is considered necessary in the clinical studies.

In the 4 and 13-week repeat-dose studies in rats, teeth toxicity was observed in the incisor, including necrosis of ameloblasts and odontoblasts and irregular dentin formation, and it did not recovered with each recovery period. Teeth changes were not observed in dogs and in the molar of rats. In general, the permanent teeth in humans and dogs or the molar in rodents do not grow, but growth of incisor is continuous in rodents. Therefore, the PROJECT N induced incisor changes in rats are probably rodent-specific and irrelevant to adult humans.

In the in vitro reverse mutation study, PROJECT N did not induce mutation. Meanwhile, PROJECT N induced numerical chromosomal aberrations in the in vitro study and micronuclei in the mouse study. However, these genotoxicity potential will not interfere with human clinical studies in advanced cancer patients.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | | **Rat: 4-week study** | | | **Dog: 4-week study** | | | **Species** | | **Rat: 13-week study** | | | **Dog: 13-week study** | | |
| **Dose (mg/kg)** | | **0.3** | **1** | **3** | **0.1** | **1** | **10** | **Dose (mg/kg)** | | **0.1** | **0.3** | **0.6** | **0.03** | **0.1** | **0.3** |
| Inorganic phosphorus (mg/dL) | | ↑  (F) | ↑  (F) | ↑  (M, F) | → | ↑  (M, F) | ↑  (M, F) | Inorganic phosphorus (mg/dL) | | → | → | ↑  (F) | → | → | ↑  (M) |
| Calcium (mg/dL) | | → | → | ↓  (F) | → | → | ↓  (M, F) | Calcium (mg/dL) | | → | → | ↓  (F) | → | → | → |
| Albumin (g/dL) | | ↓  (F) | ↓  (F) | ↓  (M, F) | ↓  (M, F) | ↓  (M, F) | ↓  (M, F) | Albumin (g/dL) | | → | ↓  (F) | ↓  (F) | → | ↓  (M, F) | ↓  (M, F) |
| PTH (pg/mL) | | ↓  (F) | ↓  (M, F) | ↓  (M, F) | ↓  (F) | ↓  (M, F) | → | PTH (pg/mL) | | → | ↓  (M) | ↓  (M, F) | → | ↓  (M) | ↓  (M, F) |
| 1,25(OH)2D (pg/mL) | | ↓  (M) | ↓  (M) | ↓  (M) | ↓  (M) | ↓  (M, F) | → | 1,25(OH)2D (pg/mL) | | → | → | → | → | ↓  (M) | ↓  (M, F) |
| Mineralization | Heart, thoracic aorta | NE | − | + (M, F) | − | + (M, F) | + (M, F) | Mineralization | Cornea, kidney | NE | − | + (M, F) | − | − | − |
| Stomach | − | + (M, F) | + (M, F) | − | − | + (M, F) | Stomach, spinal cord | NE | − | + (F) | − | − | − |
| Lung | − | − | − | − | − | +  (M, F) |

↑: Increased, ↓: Decreased, →: No change, +: Present, −: Absent, NE: Not examined; PTH: parathyroid hormone

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Rat: 4-week study** | | | **Dog: 4-week study** | | | **Rat: 13-week study** | | | **Dog: 13-week study** | | |
| **Dose (mg/kg)** | **0.3** | **1** | **3** | **0.1** | **1** | **10** | **0.1** | **0.3** | **0.6** | **0.03** | **0.1** | **0.3** |
| BAP (IU/L) | ↑  (F) | ↑  (M, F) | ↑  (M, F) | NA† | NA† | NA† | ↑  (F) | ↑  (F) | ↑  (F) | NA | NA | NA |
| TRACP 5b (U/L) | ↑  (M) | ↑  (M, F) | ↑  (M, F) | NA | NA | NA | ↑  (M) | ↑  (M, F) | ↑  (M) | NA | NA | NA |
| CS846 (ng/mL) | → | → | → | → | ↑  (M, F) | ↑  (M, F) | → | → | → | → | ↑  (M, F) | ↑  (M, F) |
| C1,2C (µg/mL) | → | → | → | ↑  (F) | ↑  (M, F) | → | → | → | → | → | → | → |
| Thickening of the cartilage | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) | + (M, F) |
| Bone decrease | − | + (F) | + (M, F) | − | − | + (M, F) | − | − | + (F) | − | − | − |

↑: Increased, ↓: Decreased, +: Present, −: Absent, NA: Not assessed

† These values were not used for an evaluation because the validation was proved unreliable in dogs.

### Table 15 Potential Safety Concern of PROJECT N

|  |  |  |
| --- | --- | --- |
| **Key Safety Targets** | **Key Observations** | **Relevance to Human Usage** |
| Hyperphosphatemia | Increased serum phosphorus and systemic tissue mineralization | Potential for hyperphosphatemia |
| Epithelial tissues | Epithelial atrophy (bronchus, cornea, esophagus, mammary gland, nail, tarsal gland, tongue) | Potential for alopecia, skin and nail disorder, stomatitis, corneal ulcer |
| Bone and cartilage | Thickening of cartilage, decreased bone | Potential for articular and bone disorder |
| Liver | Increased AST, ALT | Potential for hepatotoxicity |
| Kidney | Increased BUN, creatinine | Potential for nephrotoxicity |
| Heart / CV (incl. QT) | Increased heart rate and blood pressure | Potential for changes in heart rate and blood pressure |
| Lung | Foam cell accumulation, mononuclear cell infiltration | Potential for respiratory disability |
| Eye | Degeneration of lens fiber | Potential for cataract |
| Hematopoietic system | Decreased reticulocytes, hypocellularity of bone marrow | Potential for anemia |
| Teeth | Necrosis of ameloblast in rat incisor (not in molar).  The change is considered rodent specific. | Unlikely in adults |
| Genotoxicity | Reverse mutation study: Negative  In vitro chromosomal aberration study: Positive (numerical aberrations)  In vivo micronucleus study: Positive | Potential for genotoxicity |
| Reproductive toxicity | No reproductive and developmental toxicity studies have been conducted. | NA |
| Carcinogenicity | No carcinogenicity study has been planned. | NA |
| *Other relevant toxicity-related information* | None | None |
| Indications for clinically relevant drug interactions | None | None |

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CV: cardiovascular; NA: not applicable