### Safety Pharmacology

A tabulated overview of nonclinical safety pharmacology studies is presented in [End-of- Text Table 1.1] and a summary of the noteworthy findings is presented in [End-of-Text Table 1.2].

### In Vitro Effects on hERG Current

The effects of PROJECT K on the hERG current were studied in hERG-transfected HEK293 cells by the whole-cell patch-clamp technique (Study Project K-PT-0001).

The compensated suppression rates of PROJECT K at the concentrations of 0.3, 3 and

30 µmol/L were -3.2%, -2.7% and 1.7%, respectively; no statistically significant difference was observed at these 3 concentrations when compared to the rate in the control group.

These results indicates that PROJECT K does not affect the hERG current in hERG-transfected HEK293 cells at concentrations of up to 30 µmol/L.

### In Vitro Effects on APD

The effects of PROJECT K on APD in isolated guinea-pig papillary muscles were studied by the glass-electrode technique under a surface-superfusing condition (Study Project K-PT-0002).

PROJECT K at concentrations of 0.3, 3 and 30 µmol/L had no effect on APD, resting membrane potential (RMP), action potential amplitude (APA) or maximum upstroke velocity (dV/dt max). These results show that PROJECT K does not affect the isolated guinea pig papillary muscle APD, RMP, APA or dV/dt max at concentrations of up to 30 µmol/L.

### In Vivo Effects on CNS

The effects of PROJECT K on the CNS and on the general activity and behavior of female rats were determined (Study Project K-PT-0003). The observational parameters of the general activity and behavior were set by the modified Irwin's method. Six female rats were used in each testing group, and PROJECT K was orally administered once to the animals at doses of 1, 10 or 100 mg/kg (free base dose). Controls were administered the vehicle, 0.5 w/v% MC aqueous solution. The general activity and behavior of the animals were observed before administration, 0.5, 1, 2, 4, 6, 8 and 24 hours after administration.

Over the 24-hour observation period, PROJECT K did not affect the general activity and behavior in any rat at any dose tested (1, 10 or 100 mg/kg).

### In Vivo Effects on CNS, Cardiovascular and Respiratory Systems

The effects of PROJECT K on the CNS, cardiovascular and respiratory system were investigated in Study Project K-PT-0004. PROJECT K suspended in 0.5 w/v% MC aqueous solution was orally administered at dose levels of 0.1, 1, 10 and 100 mg/kg of PROJECT K (5 mL/kg dose volume) as a single oral dose to conscious male dogs.

PROJECT K did not affect the body temperature, blood pressure, heart rate, electrocardiogram, respiration rate, blood gases or blood-electrolyte concentration at doses of 0.1, 1, 10 or

100 mg/kg. However, vomiting was noted in 1 animal and occult blood was observed in the feces of all animals at the 10 mg/kg dose level; while in the 100 mg/kg dose group, vomiting was noted in 1 animal and occult blood was noted in all animals. In addition, at the 100 mg/kg dose level, compound-colored feces was observed in all animals.

Toxicokinetic assessments showed a linear dose proportional increase in both Cmax and AUC24 over a dose range of 0.1 to 10 mg/kg; while at higher doses, the Cmax tended to plateau (increased 18% with a dose increase of 10-fold). The AUC24 also increased linearly and in a dose-related manner from 0.1 to 10 mg/kg then tended to plateau from 10 to 100 mg/kg (increased 43% while the dose increased 10-fold).

These results indicate that under the conditions of this study, PROJECT K has no discernible effect on either the cardiovascular or respiratory system at doses of up to 100 mg/kg. GI effects were noted at the 10 mg/kg dose level in the form of vomiting and fecal occult blood. At higher doses (100 mg/kg), compound-colored feces were observed.

## Toxicology

A total of 15 toxicology studies were conducted in rats, dogs, and rabbits as part of the PROJECT K development program. All pivotal studies were performed in accordance with GLP standards and guidelines of the ICH. For in vivo studies, PROJECT K was administered orally as a 0.5% MC solution/suspension. For in vitro studies, PROJECT K was dissolved in dimethylsulfoxide and applied in medium.

A tabulated overview of the nonclinical toxicology studies is presented in [End-of-Text Table 3.1]. A tabulated overview of the nonclinical toxicokinetic studies and the toxicokinetic data are presented in [End-of-Text Tables 3.2 and 3.3], respectively.

### Single-dose Toxicity

A summary of the noteworthy findings from the single-dose toxicity studies is presented in [End-of-Text Table 3.4].

### Rats

PROJECT K was administered orally once at dose levels of 500, 1000 and 2000 mg/kg to 5 male and 5 female rats per group in order to investigate its toxicity (Study Project K-TX-0014).

Animals were necropsied on the following day of a 14-day observation period.

No animal died and no test article-related changes were noted in clinical signs or gross pathology in any group. A slight loss of body weight occurred in 2 females in the

1000 mg/kg group on day 2 or day 10. The body weights of the other animals increased satisfactorily throughout the observation period. Under the conditions of this study, the approximate lethal dose was greater than 2000 mg/kg for males and females.

### Dogs

PROJECT K was given as a single oral dose of 500 or 1500 mg/kg in a dose-volume of

15 mL/kg to dogs (1/sex/group) in order to examine its toxicity (Study Project K-TX-0016). Animals were necropsied on the following day of a 14-day observation period.

No deaths occurred in any treatment groups. At 500 mg/kg, treatment-related changes were as follows: vomiting, grayish white or reddish feces, increases in reticulocyte ratio, fibrinogen and alanine transaminase (ALT), decrease in serum calcium, and extramedullary hematopoiesis in the spleen. At 1500 mg/kg, the dogs showed vomiting and grayish white feces or (dark) red feces of normal, soft or watery consistency. A decrease in food consumption and a decrease in levels of total protein, albumin, total bilirubin, ALT and calcium in serum were noted. The reticulocyte ratio and severity of extramedullary hematopoiesis in the spleen were increased. Additionally, increases in white blood cell (WBC), neutrophils and monocyte counts, levels of fibrinogen and blood urea nitrogen (BUN), potassium and chloride were observed.

These changes generally had disappeared from 9 days after dosing. The changes in hematology and blood chemistry had recovered or were recovering by the end of a 14-day observation period, except for the increases in the reticulocyte ratio (in the female at 500 and 1500 mg/kg) and the increased levels of fibrinogen and potassium (in the female at

1500 mg/kg), which were still present 14 days after the end of dosing.

In toxicokinetics, Cmax and AUC24 values showed a non-proportional dose-related increase and no sex differences in exposure.

Under the conditions of this study, the lethal dose in dogs was greater than 1500 mg/kg. Treatment with PROJECT K induced fecal blood loss, and it is hypothesized that there was a compensatory increase in hematopoiesis as evidenced by an increased reticulocyte ratio and enhanced extramedullary hematopoiesis in the spleen.

### Repeat-dose Toxicity

A summary of the noteworthy findings from the nonpivotal repeat-dose toxicity studies is presented in [End-of-Text Table 3.5]. Tabulated results of the pivotal repeat-dose toxicity studies are presented in [End-of-Text Table 3.6].

### Rats

* + - * 1. **1-week Oral Toxicity Study (Exploratory)**

PROJECT K (free base) was administered orally to 5 male and 5 female rats once daily for 7 days at doses of 0 (vehicle), 0.1, 1, 10 and 300 mg/kg per day (Study Project K-TX-0001).

No animal died during the dosing period. No test article-related changes were noted in any group in clinical signs, body weight, food consumption, hematology, blood chemistry, gross pathology and organ weight. In males, at the highest dose tested (300 mg/kg/day) and in females at doses of 10 or 300 mg/kg per day, erosion, mucosal and submucosal inflammatory cell infiltration, basophilic mucosal epithelial cells and peritonitis in the cecum were observed in histopathology.

In toxicokinetics, the Cmax and AUC24 values increased with the dose level; however, the values increased less than dose-proportionally. The Cmax and AUC24 values in females were higher than those in males at each dose. The values on day 7 were similar to those on day 1, except for AUC24 in females at 1 mg/kg per day.

The NOAEL, under the condition of this study, was considered to be 10 mg/kg per day for males and 1 mg/kg per day for females.

### 1-week Oral Toxicity Study, Additional High Dose (Exploratory)

PROJECT K (free base) was administered orally to 5 male and 5 female rats once daily for 7 days at doses of 0 (vehicle) and 1000 mg/kg per day (Study Project K-TX-0002).

No animal died during the dosing period. No test article-related changes were noted in any group in clinical signs, body weight, food consumption, hematology and organ weight. At 1000 mg/kg per day, gross pathological examination revealed dark-red spots in the glandular stomach in both males and females. In histopathology, erosion in the glandular stomach was observed in males and females. In females, submucosal inflammatory cell infiltration and peritonitis in the cecum were noted. In addition, blood chemistry testing indicated increases in aspartate aminotransferase activity and BUN level in females.

In toxicokinetics, the Cmax and AUC24 values in the 1000 mg/kg per day group were greater than those in the 300 mg/kg per day group in the previous exploratory study. The AUC24 values at 1000 mg/kg per day in Study Project K-TX-0002 were approximately 1.5 to 3 times higher than those of 300 mg/kg per day in Study Project K-TX-[0001 [Section 4.3.2.1.1](#_bookmark53)]. The Cmax and AUC24 values in females were higher than those in males at 1000 mg/kg per day. The values on day 7 were similar to those on day 1 in both sexes.

### 4-week Oral Toxicity Study (Definitive)

From the results of toxicokinetic bridging study (Project K-TX-0005), 100 mg/kg per day was used as the highest dose in Study Project K-TX-0013 because the plasma drug concentration was thought to reach maximum at the dose of 100 mg/kg per day. Therefore, PROJECT K was orally administered once daily for 4 weeks at dose levels of 0 (vehicle), 0.1, 1, 10 and

100 mg/kg per day to 10 male and 10 female rats per group in order to investigate its toxicity

(Study Project K-TX-0013). Five males and five females were added to the control and the highest dose groups to assess the reversibility of toxicity during a subsequent 4-week recovery period. A satellite group (3 males and 3 females in the control group, and 9 males and 9 females in each test-article group) was added at each dose level to assess systemic exposure to PROJECT K.

During the dosing and recovery periods, no animal died and no toxic changes were noted in clinical signs, body weight, food consumption or ophthalmology at any dose level.

At 0.1 mg/kg per day, in both males and females, erosion/ulcer and inflammatory cell infiltration in mucosa and submucosa in the glandular stomach were observed. In females, decreased urine volume, increased urinary specific gravity, dark red spots or foci in the glandular stomach, and inflammatory cell infiltration in mucosa and submucosa in the cecum were noted.

At 1 mg/kg per day, in both males and females, erosion/ulcer and inflammatory cell infiltration in mucosa and submucosa in the glandular stomach were noted. In females, tendencies to decrease urine volume and increase urinary specific gravity, erosion/ulcer and inflammatory cell infiltration in mucosa and submucosa in the cecum were found.

At 10 mg/kg per day, in both males and females, erosion/ulcer and inflammatory cell infiltration in mucosa and submucosa in the glandular stomach and the cecum were seen. In females, decreased urine volume, a tendency to increase urinary specific gravity and dark red foci in the glandular stomach were observed.

At 100 mg/kg per day, in males and females, a tendency to increase in neutrophil count, decreases in total protein, globulin and calcium levels, dark red spots in the glandular stomach, erosion/ulcer and inflammatory cell infiltration in mucosa and submucosa in the glandular stomach and the cecum were observed. In females, a tendency to decrease urine volume, increased urinary specific gravity, increased reticulocyte ratio, decreased albumin level, as well as increased absolute and relative spleen weights were detected.

The above findings were not detected in the 4-week recovery group, although fibrosis, which indicated the repair of mucosal injury, was noted in the cecum in the recovery 100 mg/kg per day group.

In toxicokinetics, throughout the dosing period, the Cmax values for both males and females increased almost in proportion to the dose levels up to 10 mg/kg per day, and at a ratio lower than the dose ratio at 100 mg/kg per day. AUC24 values for both males and females increased almost dose-proportionally throughout the dosing period. The Cmax or AUC24 values on day 1 were not much different from those of the treatment group in week 2 or 4.

The Cmax and AUC24 values for females in each of the treatment groups were 1.9 to 7.7 times higher than those for males, except for the Cmax values in week 2. The tmax values were 0.5 to 2 h for each of the treatment groups.

Under the conditions of the study, the NOAEL was therefore concluded to be less than

0.1 mg/kg per day for males and females because mucosal damages in the stomach and

cecum in both males and females and decreased urine volume and increased urinary specific gravity in females were noted at 0.1 mg/kg per day and greater.

### 4.3.2.1.4 4-week Oral Toxicity Study, Additional Low Dose (Definitive)

PROJECT K was orally administered once daily for 4 weeks at dose levels of 0 (vehicle), 0.003,

0.01 and 0.03 mg/kg per day to 10 male and 10 female rats per group (Study Project K-TX-0020). A satellite group (3 males and 3 females in the control group, and 18 males and 18 females in each test-article group) was added at each dose level to assess systemic exposure to PROJECT K. Considering the results of the previous study, the following examinations were performed in this study: general signs, body weight, food consumption, fecal occult blood test, urinalysis, gross pathology, histopathology and toxicokinetics.

There were no changes thought to be attributable to PROJECT K in males or females at

0.003 and 0.01 mg/kg per day, and males at 0.03 mg/kg per day. The changes thought to be attributable to PROJECT K were an increase in urine specific gravity and decreasing trend in urine volume in females at 0.03 mg/kg per day.

The Cmax and AUC24 values increased almost dose-proportionally through the dosing period in both sexes. These values in all groups were almost constant throughout the dosing period. The tmax values in all treatment groups were 1 h, except for in females at 0.03 mg/kg per day on week 2 (at 0.5 h).

The NOAEL in this study was therefore concluded to be 0.03 mg/kg per day for males and

0.01 mg/kg per day for females.

### Dogs

* + - * 1. **1-week Oral Toxicity Study (Exploratory)**

PROJECT K (free base) was administered orally to 1 male and 1 female dog once daily for

7 days at doses of 0.1, 3 and 30 mg/kg per day (Study Project K-TX-0003). Systemic exposure to PROJECT K was also evaluated.

No animal died or was sacrificed due to moribund condition in any group. No test article- related changes were noted in any group in body temperature, body weight, food consumption, ophthalmology, hematology and organ weights, nor were noted in any observation or examination in the 0.1 mg/kg per day group.

At 3 mg/kg per day, blackish feces (positive for fecal occult blood test), decrease in total protein, albumin and calcium were observed in the male and female and mucous feces was observed in the male. Gross pathology revealed red foci in the duodenal and ileal mucosa for the female. In histopathology, erosion in the duodenum and erosion and hemorrhage in the ileum were observed in the male and female.

At 30 mg/kg per day, blackish feces (positive for fecal occult blood test) was observed in the male and decrease in total protein, albumin and calcium was observed in the male and female. In histopathology, erosion in the duodenum was observed in the male and female.

In toxicokinetics, the Cmax and AUC24 values increased with the increasing dose level. No apparent sex differences were noted. The values on day 7 were similar to those on day 1 at all doses.

The NOAEL was considered to be 0.1 mg/kg per day for both males and females.

### 2-week Oral Toxicity Study (Exploratory)

PROJECT K was administered orally to 1 male and 1 female dog once daily for 14 days at doses of 0.3, 3, 100 and 1000 mg/kg per day (Study Project K-TX-0007).

No animal died or was sacrificed due to moribundity in any group. No test article-related changes were noted in any group in ophthalmology.

At 0.3 mg/kg per day, decrease in mean corpuscular hemoglobin concentration (MCHC) was noted in the male and female. Inflammatory cell infiltration in the duodenal mucosa was observed in the male and vomiting, abnormal color feces (reddish feces, reddish soft feces, dark red feces and dark red soft feces), decrease in total protein, albumin, globulin, total bilirubin, total cholesterol, alkaline phosphatase (ALP) activity, ALT activity and calcium, regeneration of the mucosal epithelial cell in the ileum and mucosal hemorrhage in the cecum were observed in the female.

At 3 mg/kg per day, abnormal color feces (reddish feces and dark red feces), decrease in total protein, albumin and calcium were observed in the male and female. Vomiting, decreased food consumption, decrease in globulin, inflammatory cell infiltration in the duodenal mucosa and regeneration in the mucosal epithelial cell in the ileum were observed in the male and decrease in hemoglobin, MCHC and ALT activity, increase in fibrinogen, WBC, neutrophil and monocyte counts and chloride, extramedullary hematopoiesis in the spleen, erosion/ulcer in the duodenum were observed in the female.

At 100 mg/kg per day, vomiting, abnormal color feces (reddish feces, reddish soft feces, dark red feces and dark red soft feces), decreased food consumption, decrease in MCHC, total protein, albumin, globulin, ALP activity, ALT activity and calcium, increase in reticulocyte ratio, hypercellularity in the sternal and femoral bone marrow, extramedullary hematopoiesis in the spleen and regeneration of the mucosal epithelial cell in the jejunum were observed in the male and female. In addition, vomitus with test substance, decrease in red blood cell (RBC) count, hemoglobin and hematocrit levels and total bilirubin, increase in chloride, regeneration of the mucosal epithelial cell in the duodenum, erosion/ulcer in the ileum were observed in the male. Increase in WBC, neutrophil and monocyte counts, decrease in total cholesterol and glucose, erosion/ulcer in the duodenum, regeneration of the mucosal epithelial cell in the ileum were observed in the female.

At 1000 mg/kg per day, abnormal color feces (male and female: reddish feces, reddish soft feces, dark red feces and dark red soft feces, male: reddish and dark red watery feces), vomiting, vomitus with test substance, grayish white feces, decreased food consumption, decrease in MCHC, total protein, albumin, globulin, ALP activity, ALT activity and calcium, increase in chloride, hypercellularity in the sternal bone marrow, extramedullary hematopoiesis, lymphoid follicular atrophy in the spleen, thymic atrophy, erosion/ulcer in the

duodenum were observed in the male and female. In addition, salivation, watery feces, grayish white soft feces, decreased body weight, increase in WBC count, decrease in glucose, increase in the absolute and relative weight of the spleen, hypercellularity in the femoral bone marrow, erosion/ulcer in the ileum, erosion in the cecum were observed in the male.

Decrease in RBC count, hemoglobin and hematocrit levels, total bilirubin and total cholesterol, fluid in the abdominal cavity (ascites), edema in the lamina propria of the stomach, erosion in the pyloric stomach, regeneration of the mucosal epithelial cell in the ileum and interstitial edema and islet atrophy in the pancreas were observed in the female.

In toxicokinetics, throughout the dosing period, the Cmax and AUC24 values for both males and females increased almost in proportion to the dose levels up to 3 mg/kg, and at a ratio lower than the dose ratio at 100 mg/kg. The Cmax and AUC24 values at 1000 mg/kg were almost equivalent to those at 100 mg/kg in males and were lower than those at 100 mg/kg in females. The Cmax or AUC24 values on day 1 were not much different from those during day 7 or 14 in any of the treatment groups, although the Cmax values on day 7 at 3 mg/kg, the

Cmax and AUC24 values on day 14 at 100 mg/kg and the Cmax and AUC24 values on day 7 and 14 at 1000 mg/kg were lower than those on day 1, respectively. No apparent sex differences were noted.

The NOAEL in this study was concluded to be less than 0.3 mg/kg per day for both sexes.

### 4-week Oral Toxicity Study (Definitive)

PROJECT K was orally administered to 4 male and 4 female dogs per group at doses of

0 (vehicle), 0.03, 0.3 and 100 mg/kg per day once daily for 4 weeks to evaluate toxicological changes (Study Project K-TX-0017). A recovery group consisting of 3 male and 3 female dogs treated with 100 mg/kg per day was also included, to investigate whether toxic changes induced during the 4 week treatment period resolved after a 4-week recovery period.

There was no animal death in any dose group. Toxicological changes were not observed at

0.03 mg/kg per day, but did occur at 0.3 mg/kg per day or more.

At 0.3 mg/kg or more per day, findings included vomiting, soft feces and abnormal-colored feces, which contained blood (reddish, reddish soft, reddish watery, and reddish mucous feces; dark-red, dark-red soft, dark-red watery, and dark-red mucous feces); increases in fibrinogen; decreases in total protein, albumin, calcium and sodium; and infiltration of inflammatory cells in the duodenal mucosa.

At 100 mg/kg per day, findings included those observed in the 0.3 mg/kg per day group, as well as the following: vomitus with the test article, watery feces and emaciation; decreases in body weight and food consumption; decreases in RBC count, hemoglobin, hematocrit, MCHC, globulin, albumin/globulin ratio, total bilirubin, total cholesterol, ALP activity, ALT activity and glucose; and increases in platelet count, reticulocyte ratio, WBC count, neutrophil count, monocyte count and chloride. Autopsy findings included depressed focus and dark red focus in the duodenum, fluid in the abdominal cavity (ascites), edema of the pancreas and obscure thymus. There were statistically significant increases in absolute and relative weights of the spleen, and relative weight of the pancreas, and statistically significant

decreases in absolute and relative weights of the thymus as compared with those of the control group. In histopathology, erosion/ulcer in the duodenum, erosion/ulcer and regeneration of mucosal epithelia in the ileum, hypercellularity in the sternal and femoral bone marrows, extramedullary hematopoiesis in the spleen, thymic atrophy, granuloma in the liver and interstitial edema of the pancreas were observed.

In the recovery group, among the changes induced during the 4-week administration period, increases in platelet count and reticulocyte ratio, and decreased MCHC were still observed. In addition, some animals had decreases in mean corpuscular volume and mean corpuscular hemoglobin. These changes were judged to be secondary to recovery of erythrocyte parameters that decreased or tended to decrease during the treatment period, and were expected to resolve by prolongation of the recovery period since the erythrocyte parameters returned to normal values by 4-week drug withdrawal. Soft feces, watery feces and abnormal-colored feces, increased relative weight of the spleen and hypercellularity in the sternal and femoral bone marrows were alleviated in incidence or severity, and thus they were considered to show tendency to recovery. No other changes occurred in the recovery group.

When the dose was increased from 0.03 to 0.3 mg/kg per day, Cmax and AUC24 values on

day 1 of males increased at a rate equal to the dose ratio (Cmax: 9.8 fold, AUC24: 9.6 fold) and those of females increased at a rate lower than the dose ratio (Cmax: 7.1 fold, AUC24:

7.5 fold). When the dose was increased from 0.3 to 100 mg/kg per day, the values for both males and females increased at a rate lower than the dose ratio (Cmax: 12.6 and 17.0 fold, respectively; AUC24: 24.2 and 17.2 fold, respectively). Comparison of the Cmax and AUC24 values between day 1 and week 2 or 4 indicated that the AUC24 value for females in the 100 mg/kg per day group at week 2 was 2.5 times higher than that on day 1. No other significant difference was observed in any group. No gender difference was noted.

The NOAEL for this study was judged to be 0.03 mg/kg per day. Changes during administration can be expected to disappear or tend to disappear by drug withdrawal.

### Genotoxicity

Tabulated results of genotoxicity studies are presented in [End-of-Text Table 3.7].

### In Vitro Reverse Mutation Study

In order to assess the potential of PROJECT K to induce gene mutation, a bacterial reverse mutation test was performed with 5 strains of bacteria (*Salmonella typhimurium* [TA100, TA1535, TA98 and TA1537] and *Escherichia coli* [WP2*uvrA*]), using the preincubation method with and without metabolic activation (Study Project K-TX-0008). Based on the results of the dose-finding test, the main test was performed at 156, 313, 625, 1250, 2500 and 5000 μg/plate as PROJECT K in all test strains with and without metabolic activation.

Test article precipitation was observed at 5000 μg/plate upon addition of the test article formulation and on the plates after incubation for 48 hours with and without metabolic activation.

Growth inhibition was observed at 5000 μg/plate in TA1537 without metabolic activation. Growth inhibition was not observed in the other test strains with and without metabolic activation.

In comparison with the negative control, a 2-fold or greater and dose-dependent increase in the number of revertant colonies was not observed in any test strain in the dose-finding test or the main test, with and without metabolic activation.

Under the conditions of this study, PROJECT K did not induce gene mutation in bacteria.

### In Vitro Chromosome Aberration Study

In order to assess the potential of PROJECT K to induce chromosomal aberrations, a chromosomal aberration test was performed with cultured fibroblast derived from the lung of female Chinese hamster (CHL/IU) cells in short-term treatments for 6 hours with and without metabolic activation, and continuous treatment for 24 hours without metabolic activation (Study Project K-TX-0010).

The dose levels for the chromosomal aberration test were set based on the results of the

dose-finding test. The lowest dose that showed a cell proliferation ratio of less than 50% was set as the highest concentration. Chromosomal aberrations were analyzed at the following doses: 267, 400, 600 and 750 μg/mL in short-term treatment with and without metabolic activation, and 79.0, 119, 178, 267 and 400 μg/mL in continuous treatment for 24 hours. The number and incidence of cells with structural and numerical chromosomal aberrations were investigated.

No significant increase in the number of cells with structural or numerical chromosomal aberrations was noted in any treatment group when compared with the negative control group.

Under the conditions of this study, PROJECT K did not induce chromosomal aberrations in CHL/IU cells, regardless of the presence or absence of metabolic activation, or treatment length.

### Carcinogenicity

No carcinogenicity studies have been conducted to date.

### Reproductive and Developmental Toxicity

Exploratory dose range-finding and definitive studies for embryo-fetal developmental toxicity were conducted in rats and rabbits.

A summary of the noteworthy findings from the nonpivotal reproductive and development toxicity studies is presented in [End-of-Text Table 3.10]. Tabulated results of the pivotal repeat-dose toxicity studies regarding the effects on embryo-fetal development are presented in [End-of-Text Table 3.12].

### Rats

* + - * 1. **Embryo-fetal Development Study (Exploratory)**

This study was designed to preliminarily evaluate the potential adverse effects of PROJECT K on pregnant animals and embryo-fetal development (embryo-fetal death, growth retardation and malformations) in rats when PROJECT K was administered orally to 6 pregnant rats at dose levels of 0 (vehicle), 100, 300 and 1000 mg/kg per day on days 7 to 17 of gestation, the days that correspond to the period from implantation to closure of the hard palate of rat fetuses (Study Project K-TX-0011).

No death occurred in any dam. A red substance in the cage tray, suppressed body weight gain and large spleen in the 100 and 300 mg/kg per day groups, deceased food consumption in the 100 mg/kg per day group and a dark reddish focus on the mucosa of the stomach in the 1000 mg/kg per day group were noted.

Increases in the postimplantation loss and decreases in the number of live fetuses and fetal body weights were noted in the 100 and 300 mg/kg per day groups. Fetal body weights tended to decrease in the 1000 mg/kg per day group, but neither increases in the postimplantation loss nor decreases in the number of live fetuses were noted in this group. No treatment-related effects were noted on the sex ratio or placental weights of live fetuses. In addition, no clear effects were noted in the morphological observations (external, visceral and skeletal).

Based on these results, it was considered to be appropriate that an additional dose-range finding study is conducted at lower dose levels than 100 mg/kg per day to select dose levels for the subsequent definitive study.

### Embryo-fetal Development Study, Additional Low Dose (Exploratory)

This study was designed to preliminarily evaluate the potential adverse effects of PROJECT K on pregnant animals and embryo-fetal development (embryo-fetal death, growth retardation and malformations) in rats when PROJECT K was administered orally to 6 pregnant rats at dose levels of 0 (vehicle), 1, 10 and 100 mg/kg per day on days 7 to 17 of gestation, the days that correspond to the period from implantation to closure of the hard palate of rat fetuses (Study Project K-TX-0012).

No death occurred in any dam. A red substance in the cage tray, suppressed body weight gain and large spleen were noted in the 100 mg/kg per day group. No treatment-related effects were noted on the number of corpora lutea or implantation or preimplantation loss.

An increase in the postimplantation loss and decreases in the number of live fetuses and fetal body weights were noted in the 100 mg/kg per day groups. No treatment-related effects were noted on the sex ratio or placental weights of live fetuses. In addition, no clear effects of the test article were noted in the external observations.

The Cmax and AUC24 values of PROJECT K increased almost dose-proportionally up to

100 mg/kg per day, excluding the AUC24 values on the first day of dosing. The AUC24 value on the first day of dosing increased more than dose-proportionally. The Cmax and AUC24 values were almost comparable between the first and last days of dosing, excluding the AUC24 values in the 1 mg/kg per day group.

Based on these results, 100 mg/kg per day was considered to be excessive to evaluate the effects of PROJECT K on embryo-fetal development. Therefore, dose levels less than

100 mg/kg per day were considered to be appropriate as a highest dose for a subsequent definitive study.

### Embryo-fetal Development Study (Definitive)

PROJECT K was administered orally to 18 to 20 pregnant rats/group at dose levels of 0 (vehicle), 1, 10, 30 and 1000 mg/kg per day on days 7 to 17 of gestation, the days that correspond to the period from implantation to closure of the hard palate of rat fetuses (Study Project K-TX-0018).

In dose-range finding studies, increase in the post-implantation losses (%) was noted at

100 and 300 mg/kg per day, while it was not observed at 1000 mg/kg per day. Therefore, the high-dose level for Study Project K-TX-0018 was set at 1000 mg/kg to confirm the dose- dependency of the finding.

No death occurred in any dam. Total litter (embryo/fetus) loss was observed in 3 dams in the 1000 mg/kg per day group. In this group, paleness of the whole body, discoloration of the eyes, blackish stools, suppressed body weight gain, decreased food consumption, a large spleen and pale discoloration of the liver were also noted in dams. In the 30 mg/kg per day group, no appreciable changes were noted in the physical condition, body weights or food consumption, but a dark reddish focus on the mucous of the stomach was observed. No changes suggestive of effects of treatment with the test article were noted in the 1 or

10 mg/kg per day group.

In the 1000 mg/kg per day group, an increase in the postimplantation loss, decreases in the number and body weights of live fetuses and a decrease in the number of ossified metacarpi were noted. In addition, the incidence of membranous ventricular septum defect was high in this group. In the 30 mg/kg per day group, although increases in fetal body weights and the number of ossified sacral and caudal vertebrae were noted, these were considered to be of no toxicological significance since these findings were not associated with morphological changes. No changes suggestive of effects of treatment with the test article were noted in the 1 or 10 mg/kg per day group. No effects of treatment with the test article were noted in the sex ratio, placental weight or incidences of skeletal malformations or variations of live fetuses in any test article treated group.

The Cmax and AUC24 values of PROJECT K increased with increasing dose levels on both days 7 and 17 of gestation. These values were nearly comparable regardless of the duration of administration. PROJECT K was not detected in plasma in any control sample.

Based on the results of this study, the NOAELs were 10 mg/kg per day for maternal general toxicity and 30 mg/kg per day for maternal reproductive function and embryo-fetal development.

### Rabbits

* + - * 1. **Embryo-fetal Development Study (Exploratory) Study in Non-pregnant Animals**

PROJECT K was administered orally to 3 non-pregnant female rabbits at each dose level of 100, 300 and 1000 mg/kg per day for 5 days to select dose levels for the subsequent phase of this study in pregnant animals (Study Project K-TX-0009).

No death occurred in any animal. Scant feces/urine or no urine was observed and a tendency to decrease in body weights and food consumption was noted in all groups. Gross pathology revealed dark reddish foci or depression on the mucosa of the digestive tract (stomach or jejunum) in all groups. The degree of these changes was increased with increasing dose levels.

On days 1 and 5 of dosing, the Cmax and AUC24 values were almost comparable across all dose groups, up to 1000 mg/kg per day.

### Study in Pregnant Animals

PROJECT K was administered orally to 4 to 6 pregnant rabbits at each dose level of 0 (vehicle), 30, 100 and 300 mg/kg per day on days 6 to 18 of gestation, the days that correspond to the period from implantation to closure of the hard palate of rabbit fetuses, to select dose levels for the subsequent main study (Study Project K-TX-0009).

No death occurred in any dam. Abortion following decreased food consumption occurred in 1 animal each in the 100 and 300 mg/kg per day groups. A tendency to increase in the incidence of scant feces/urine or no urine was noted in the 100 and 300 mg/kg per day groups. Decreased food consumption and suppression of body weight gain were noted in all treated groups and depression on the mucosa of the stomach was observed in the 300 mg/kg per day group.

Increased postimplantation loss and decreased numbers of live fetuses were noted in all treated groups. No treatment-related effects were noted on the sex ratio, body weights, placental weights or external or visceral morphology of live fetuses. In the skeletal examinations for the 300 mg/kg per day group, no appreciable changes exceeding the historical values were noted in the skeletal malformations, variations or ossification.

Based on these results, 30 mg/kg per day, a dose at which maternal and fetal-embryonic toxicity would be expected, was considered to be appropriate as the highest dose level for the subsequent definitive study.

### Embryo-fetal Development Study (Definitive)

PROJECT K was administered orally to 18 to 19 pregnant rabbits at each dose level of 0 (vehicle), 0.3, 3 and 30 mg/kg per day on days 6 to 18 of gestation, the days that

correspond to the period from implantation to closure of the hard palate of rabbit fetuses (Study Project K-TX-0015).

No death occurred in any dam. Abortion occurred in 1 dam each in the control and 30 mg/kg per day group. Decreased food consumption, scant feces at a high incidence and suppressed body weight gain were noted in dams in the 3 and 30 mg/kg per day groups. Gross pathology of dams revealed a depression in the mucosa of the digestive tract (stomach) in the 30 mg/kg per day group. No treatment-related effects were noted on the maintenance of pregnancy of dams.

Increased post-implantation loss and decreased number of live fetuses were noted in the 3 and 30 mg/kg per day groups. Increases or a tendency to increase were noted in the

incidences of external malformations of live fetuses in the 3 and 30 mg/kg per day groups. Increases or a tendency to increase were noted in the incidences of skeletal malformations of live fetuses in the 0.3, 3 and 30 mg/kg per day groups. No treatment-related effects were noted on the sex ratio, fetal body or placental weights, incidence of visceral malformations or skeletal variations or the number of ossified bones of liver fetuses.

The Cmax and AUC24 values of PROJECT K increased with increasing dose on both days 6 and 18 of gestation. The Cmax and AUC24 values in the 30 mg/kg per day group on day 18 of gestation were about 3 and 2 times higher than those on day 6 of gestation, respectively.

Based on the results of the study, the NOAELs were concluded to be 0.3 mg/kg per day for general toxicity in dams, 30 mg/kg per day for reproductive function of dams and less than

0.3 mg/kg per day for embryo-fetal development.

### 4.3.5.2.3 Embryo-fetal Development Study, Additional Low Dose (Definitive)

Study Project K-TX-0019 was conducted in order to determine the NOAEL for fetuses. PROJECT K was administered orally to 17 to 20 pregnant rabbits at each dose level of

0 (vehicle), 0.03 and 0.1 mg/kg per day on days 6 to 18 of gestation, the days that correspond to the period from implantation to closure of the hard palate of rabbit fetuses.

No death or abortion occurred in any dam. No treatment-related effects were noted on physical condition, body weights, food consumption or gross pathology of dams as well as the maintenance of pregnancy of dams. No treatment-related effects were noted on the viability or growth of embryos/fetuses or external, visceral or skeletal morphology of live fetuses.

The Cmax and AUC24 values of PROJECT K increased with increasing dose on both days 6 and 18 of gestation. The Cmax and AUC24 values in the 0.1 mg/kg per day group on day 18 of gestation were about twice higher than those on day 6 of gestation.

Based on the results of this study, the NOAEL was concluded to be 0.1 mg/kg per day for embryo-fetal development.

### Local Tolerance

No local tolerance studies have been completed to date.

### Other Toxicity Studies

A summary of the noteworthy findings from other toxicity studies is presented in [End-of- Text Table 3.16].

### 4.3.7.1 In Vitro Phototoxicity Study

In order to investigate the potential phototoxicity of PROJECT K, a phototoxicity study was performed with cultured mammalian cells (Balb/c 3T3 cells) (Study Project K-TX-0022). A dose range-finding test was performed at 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 μg/mL as PROJECT K in the presence and absence of ultraviolet (UV)-A irradiation. The IC50 for cell viability could not be determined in either the presence or absence of irradiation because 50% or greater cytotoxicity was not observed at up to the highest non-precipitating dose, although a dose-dependent decrease in the cell viability was observed. The results for cell viability at 500 μg/mL and greater were not evaluated because test article precipitation was observed at 1000 μg/mL at the start of treatment, but was observed at 500 μg/mL and greater at the end of treatment in the presence and absence of irradiation. Therefore, the main test was performed at 37.9, 53.1, 74.4, 104, 146, 204, 286 and 400 μg/mL as PROJECT K in the presence and absence of UV-A irradiation.

No test article precipitation in the treatment mixture was observed at up to 400 μg/mL at the start or end of treatment.

The result was judged from the mean photo effect because the IC50 for cell viability could not be determined in either the presence or absence of irradiation. The mean photo effect (actual value: -0.004) was less than 0.1. Therefore, PROJECT K was categorized as having no phototoxic potential.

Under the conditions of this study, PROJECT K showed no potential to induce phototoxicity to cultured mammalian cells (Balb/c 3T3 cells).

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

Available pharmacological data show that PROJECT K has antagonistic activity on the

EP4 receptor. PGE2 binds to EP receptors, which are classified into 4 subtypes: EP1, EP2, EP3 and EP4 receptors. The EP4 receptor plays an important role in the kidney.

EP4 receptors are known to be present at podocytes, juxtaglomerular apparatus and afferent arteriole in the kidney where they would play important roles in albumin permeability and regulation of glomerular hemodynamics. These roles of EP4 receptors in the kidney suggest that EP4 receptor antagonism is of relevance for CKD treatment.

The affinity of PROJECT K on rat and human EP4 receptor was determined with Ki values of

6.02 and 2.21 nmol/L, respectively. Antagonistic activity of PROJECT K on responses mediated by rat/human EP4 receptor was determined by a PGE2-mediated cAMP accumulation assay, with IC50 values of 0.86 and 0.29 nmol/L, respectively. PROJECT K had less than 50% antagonistic activities against rat EP1, EP2 or EP3 receptors, up to

1000 nmol/L, as measured by PGE2-mediated cAMP accumulation (for EP2

receptor-expressing CHO cells) or calcium mobilization (for EP1 or EP3 receptor-expressing HEK293 cells). In addition to human CRTH2, human D prostanoid receptor and human EP2/EP3 receptors, PROJECT K at 1 μmol/L did not inhibit other 46 receptors, 5 ion channels, 3 transporters or 3 enzyme reactions by more than 50%.

The Cmax and AUC24 of PROJECT K in the repeated dose toxicity studies in rats and dogs increased with doses. There were no consistent changes after 4-weeks repeated dosing on systemic exposure, suggesting a low possibility of auto-induction of metabolism/elimination. The Cmax and AUC24 values for the female rats in each treatment group were 1.8- to 7.7-times higher than those for the males, except for the Cmax values in week 2.

There are species differences in terms of absolute bioavailability for PROJECT K (46% - 61% for rats and 78% - 88% for dogs). Radioactivity data and pharmacokinetic curves of rats show a probable enterohepatic circulation effect in contrast to dogs. Plasma protein binding was comparable between rat (~ 89%) and dog (~ 88%), but slightly higher in monkey

(~ 93%) and human plasma (91% - 96%). In human plasma, HSA and AGP were found to be the main binding proteins for PROJECT K.

After a single oral administration of [14C]PROJECT K to rats, PROJECT K was extensively metabolized. It was mainly excreted in the feces (85.6% of the dose), most of which is via the bile. In the metabolism study using human hepatocytes, at least 3 metabolite peaks were detected, but no human specific metabolites were formed. One of the metabolite peaks formed by human hepatocytes was *N*-dealkylated PROJECT K. It is currently not known whether this metabolite is pharmacologically active and it is yet to be studied which enzyme(s) contribute to the formation of this metabolite. Another one of the other human metabolites is assumed to be the acyl glucuronide of PROJECT K.

Inhibition of the major CYP isoenzymes by PROJECT K was minimal up to 50 μmol/L. For CYP3A4, a time-dependent inhibition by PROJECT K was shown. PROJECT K does not inhibit P-gp up to 70 μmol/L. PROJECT K is a P-gp substrate.

Assessment of the safety profile suggests that GI toxicity is a potential human adverse response as the GI tract was identified as a target organ for PROJECT K in rats, dogs and rabbits [[Table 6](#_bookmark66)]. In rats, the affected sites were mainly the stomach and cecum, and mucosal damages such as erosion/ulcer and inflammatory cells infiltration were observed in all repeated-dose toxicity studies. In the 1-week repeated-dose studies, cecal mucosal damages were observed in males at only 300 mg/kg and in females at 10 mg/kg or more. In addition, the gastric mucosal damages were observed in both sexes at 1000 mg/kg. In the 4-week repeated-dose study, the frequency of GI tract injuries increased due to the extended treatment period; and mucosal damages in the stomach and cecum were observed at the lower dose of 0.1 mg/kg or more in both males and females. In dogs, the affected sites were mainly duodenum and ileum, and mucosal damages presented as erosion/ulcer and inflammatory cell infiltration. In the 1-, 2- and 4-week repeated dose studies, the GI tract injuries were observed at 0.3 (inflammatory cell infiltrations), 3, 30 and 100 mg/kg (erosions/ulcers) in both sexes. These GI tract erosions/ulcers and/or inflammatory cell infiltrations were frequently associated with the clinical signs including vomiting or

abnormal color feces (reddish/dark red; soft/watery). The GI tract injuries and most of the changes associated with the GI tract injuries recovered after the 4-week recovery period. In rabbits, gross pathology of a dam revealed a depression in the mucosa of the stomach at 30 mg/kg in the ICH-3 study. Although this gross pathological lesion was limited to 1 dam, this was considered to be treatment-related since the effects of PROJECT K on the GI tract were also noted in other animal species such as rats and dogs.

Limited preclinical information is available on hemodynamics with or without angiotensin- converting enzyme inhibitor (ACEi)/angiotensin-receptor blocker (ARB). A small (acute) reduction of GFR was observed in rats (STZ hyperfiltration model without ACEi/ARB) with no effects on MBP and RBF. Monitoring of patients with CKD in the first study in patients after first dosing is advised, starting with mild CKD followed by moderate and severe CKD.

There are no indications for genotoxicity or effects on dams in the embryo-fetal development toxicity studies; however, the incidences of skeletal malformations in live fetuses were increased at 0.3 mg/kg or more in rabbits. The NOAEL for fetotoxicity in rabbits was identified as 0.1 mg/kg. There is no objection to include women of childbearing potential in the clinical studies of PROJECT K under standard contraceptive precautions, provided they use double barrier protection [[Table 6](#_bookmark66)].

### Table 6 Summary of Potential Safety Concerns

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| --- | --- |
| **Potential Safety Concern (from nonclinical studies)** | **Relevance to Human Usage** |
| GI tract   * Erosion/ulcer in GI tract including stomach, duodenum, ileum and cecum * Blood in feces | * Clinical assessment of symptoms related to the GI tract * Fecal occult blood test monitoring * Endoscope monitoring |
| Reproduction   * Embryotoxicity (post-implantation loss) * Indications for teratogenicity | * Women of childbearing potential not using adequate contraception should not be included in   development program |

GI: gastrointestinal

The NOAELs in the 4-week oral toxicity studies in rats and dogs were 0.01 mg/kg per day and 0.03 mg/kg per day, respectively. Human equivalent doses (HED) based on body surface area conversion of these NOAELs in rats and dogs are 0.0016 mg/kg and 0.016 mg/kg, respectively (FDA, 2005). The lowest HED is used for the calculation of maximum recommended starting dose for first administration to humans based on safety findings in toxicity studies (MRSDtox). The safety findings do not indicate the need for additional safety factors. Based on this assessment, the MRSDtox was calculated to be

0.0097 mg/subject.

The recommended starting dose for the first-in-human study is 0.01 mg/subject.

#### List of References

FDA/CDER Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. July 2005.