### Safety Pharmacology

* + - 1. **In Vitro Effects on hERG Current**

The effects of PROJECT Y (0, 1×10−7, 1×10−6, and 1×10−5 mol/L) on the hERG current were studied in hERG-transfected HEK293 cells using the whole-cell patch-clamp technique (Study Project Y-PT-0001).

At the highest concentration (1×10−5 mol/L), a statistically significant 13.1% reduction of hERG current was measured. The results show that PROJECT Y does not affect the hERG current in hERG transfected HEK293 cells at least up to 1×10−6 mol/L, while showing minor inhibition at 1×10−5 mol/L.

### In Vitro Effects on Action Potential Duration

The effects of PROJECT Y (0, 1×10−7, 1×10−6, and 1×10−5 mol/L) on action potentials in isolated guinea-pig papillary muscles were studied using the glass-electrode technique under a surface-superfusing condition (Study Project Y-PT-0002).

PROJECT Y did not affect the action-potential duration (APD), resting membrane potential, or any other action potential parameters in this study. These results indicate that PROJECT Y does not affect the action potentials in isolated guinea pig papillary muscles up to the highest concentration of 1×10−5 mol/L.

### In Vivo Effects on Cardiovascular and Respiratory Systems

The effects of PROJECT Y on the cardiovascular and respiratory systems were evaluated after single oral administration of 0, 10, 100, and 1000 mg/kg to 4 conscious telemetered male beagle dogs (Study Project Y-PT-0004). Effects on general activity and behavior, body temperature, blood pressure, heart rate, ECG, respiration rate, blood gases, and

blood-electrolyte concentration were investigated. Plasma drug concentration was also determined. PROJECT Y did not affect the body temperature, ECG, respiration rate, and blood-electrolyte concentration at any tested dose. At 1000 mg/kg, grayish stools (mixed

with test substance) were observed in all animals. A decreased blood pressure was observed 1 to 2 h after administration of 1000-mg/kg. Increased heart rate was observed 0.5 to 1 h after 100- and 1000-mg/kg administration. For the toxicokinetic parameters, at 10, 100 and 1000 mg/kg, the mean Cmax values were 4565, 13821 and 38497 ng/mL; the mean AUC24 values were 35507, 191705 and 336792 ng·h/mL, respectively.

In conclusion, PROJECT Y has no effect on the respiratory system at all tested doses. Statistically significant increases in heart rate and decreases in blood pressure were observed at ≥ 100 and 1000 mg/kg, respectively.

### In Vivo Effects on Central Nervous System

PROJECT Y was orally administered once at 0, 10, 100, and 1000 mg/kg to 6 male

Sprague-Dawley rats/group and effects on the general activity and behavior were assessed by a modified Irwin’s method (Study Project Y-PT-0003).

PROJECT Y did not affect the general activity and behavior in any of the rats up to 24 h after administration at any tested dose. This indicates that PROJECT Y has no effect on the central nervous system at doses up to 1000 mg/kg.

## Toxicology

A total of 26 toxicology studies were conducted in rats, dogs, mice and rabbits as part of the PROJECT Y development program. All pivotal studies were performed in accordance with GLP standards and guidelines of the ICH. For in vivo studies, PROJECT Y solid dispersion (PROJECT Y: hypromellose = 1:1) was suspended in water for injection and administered orally. For in vitro studies, PROJECT Y was dissolved in DMSO and applied in medium. The dose levels and plasma drug concentration levels were expressed as the active moiety (PROJECT Y).

### Single-dose Toxicity

* + - 1. **Rat**

Study Project Y-TX-0003 (GLP compliant) was conducted to evaluate the potential toxicity of PROJECT Y following a single oral dose in rats. PROJECT Y was orally administered once to Sprague Dawley rats (5/sex/group) at 500, 1000, and 2000 mg/kg. General signs were observed daily and body weight was measured periodically. Gross pathological examination was performed on the fourteenth day after dosing. There were no deaths in any treatment group. In addition, no test article-related findings in general signs or gross pathology were seen. A slight body weight decrease or suppression of body weight gain occurred in females in the 500-, 1000-, and 2000-mg/kg groups. The minimal lethal dose was more than

2000 mg/kg in male and female rats.

### Dog

Study Project Y-TX-0004 (GLP compliant) was conducted to evaluate the potential toxicity of PROJECT Y following a single oral dose in dogs. PROJECT Y was orally administered once at 500 or 2000 mg/kg to beagle dogs (1 animal/sex/group). Animals were observed for 14 days after administration, during which general signs were observed, and body weight and food consumption were measured. The animals underwent hematological examination, blood chemistry testing, and toxicokinetic analysis. No deaths occurred. Vomiting and yellowish white feces (containing test material) were noted at 500 and 2000 mg/kg. In addition, decreased food consumption and changes in hematological and blood chemical parameters (decreases in the basophil count, total protein, albumin, triglycerides, and calcium levels and increased neutrophil count, total bilirubin, aspartate aminotransferase [AST] activity, and urea nitrogen levels) and lack of defecation (female, day 1) occurred at 2000 mg/kg. All of these changes recovered within the 14-day observation period. The minimal lethal dose was more than 2000 mg/kg in male and female dogs.

### Repeat-dose Toxicity

* + - 1. **1-Week Oral Dose Range-finding Study in Rats (Exploratory)**

Study Project Y-TX-0017 (not GLP compliant) was conducted to evaluate the potential toxicity of PROJECT Y following daily oral administration in Sprague Dawley rats for 1 week. PROJECT Y was orally administered once daily for 1 week to Sprague Dawley rats (4/sex/group) at 0, 1, 10, 100, and 1000 mg/kg. Items examined in this study included mortality, clinical signs, body weight, food consumption, clinical pathology (hematology and clinical chemistry), and pathology (gross pathology, organ weight, and histopathology). Plasma drug concentrations were determined after dosing on day 1 and day 7.

At 10 mg/kg or higher, clear pharmacology related effects were observed, which were similar to those observed in the 4-week study. At ≥ 100 mg/kg, a slight increase in total cholesterol was found in males and a slight increase in urea nitrogen in females. At 1000 mg/kg, solid dosing material in the lumen of the stomach was observed in both sexes. Effects on the stomach (red dot in the glandular stomach, swelling of the mucous neck cells, and edema and hemorrhage in the submucosa of the fundic gland) might have been caused by physical damage due to the solid dosing material in the lumen. In addition, hypertrophy of the chromophobic cells in the pars distalis of the pituitary gland and edema and hemorrhage of the submucosa in the fundic gland of the stomach were observed in males. In females, a slight increase in inorganic phosphorus and decreased pituitary weight were observed.

The plasma unchanged drug concentrations after single and repeated administrations increased with the increasing dose. The Cmax and AUC24 values after repeated dosing were approximately equal to those after a single dose, except in the 1000-mg/kg males. The Cmax and AUC24 values in females were higher than those in males except for the 1000-mg/kg group on day 7.

The NOAEL was considered to be 10 mg/kg for both sexes due to a slight increase in total cholesterol in the 100 mg/kg males and a slight increase in urea nitrogen in the 100 mg/kg females.

### 4-Week Repeated Oral Dose Toxicity Study in Rats

Study Project Y-TX-0005 (GLP compliant) was conducted to evaluate the potential toxicity of PROJECT Y when repeatedly administered as PROJECT Y solid dispersion orally to rats once daily for 4 weeks, and to investigate the reversibility of toxicity after a 4-week recovery period.

Systemic exposure to PROJECT Y was also investigated. PROJECT Y was orally administered once daily for 4 weeks at 0, 1, 10, 100, and 1000 mg/kg to Sprague Dawley rats (10/sex per group). The observations and examinations included clinical signs, body weight, food consumption, ophthalmology, urinalysis, hematology, blood chemistry, gross pathology, organ weight and histopathology. Additional animals were included for assessment of reversibility of toxicity during 4-week recovery and for toxicokinetic and testosterone analysis.

No animals died in any group.

Atrophic changes in the genital organs in males and females and decreased testosterone concentrations in males observed at all doses of PROJECT Y were considered as pharmacology rather than toxicology related findings. In addition, the variation in body weight, anemic changes in males, and low pituitary weight were also considered to be pharmacological effect-related.

At ≥ 100 mg/kg, high thymus weight was noted in males and females. Shortened prothrombin time and activated partial thromboplastin time (aPTT), high total cholesterol, hypertrophy of the zona fasciculata cells in the adrenals were noted in males. Prolonged prothrombin time and low total protein and albumin concentration were noted in females. At 1000 mg/kg, pale yellowish white solid material (study drug) in the gastric lumen was observed in males and females. High adrenals weight, red foci in the gastric glandular mucosa, and hemorrhage in the lamina propria in the stomach were noted in males. High urine volume, prolonged aPTT, low albumin and albumin/globulin (A/G) ratios in protein fraction, and high serum inorganic phosphorus were noted in females. During and at the end of the 4-week recovery period at 1000 mg/kg, some of the pharmacological effect-related changes in the genital organs were still observed; however, they showed a tendency toward recovery. Other changes noted during the dosing period disappeared or tended to recover.

In toxicokinetics, Cmax and AUC24 increased more than dose-proportionally up to

1000 mg/kg, except for Cmax values on days 14 and 28 of dosing in females at 1000 mg/kg.

Cmax values on days 14 and 28 of dosing in females at 1000 mg/kg increased almost

dose-proportionally. Cmax and AUC24 tended to be almost constant during the dosing period. Systemic exposure in females was slightly greater than in males, except at 1000 mg/kg on days 14 and 28 of dosing.

The NOAEL was 10 mg/kg per day. Changes noted during the dosing period disappeared or tended to recover during the 4-week recovery period.

### 26-Week Repeated Oral Dose Toxicity Study in Rats

Study Project Y-TX-0019 (GLP compliant) was conducted to evaluate the toxicity profile of PROJECT Y solid dispersion, when it was administered orally by gavage to rats once daily for 26 weeks. Drug concentrations in plasma were determined to examine systemic exposure to PROJECT Y. Testosterone concentrations in serum were determined in the male animals only.

PROJECT Y was orally administered once daily for 26 weeks at 0, 1, 10, 100, and 1000 mg/kg to Sprague Dawley rats, age 7 weeks at start of administration (12/sex per group). In order to determine drug concentrations in plasma to assess toxicokinetics and testosterone concentrations in serum (only for males in week 26 of administration for testosterone), satellite groups consisting of 13 males and 7 females for the control group and consisting of 13 males and 13 females for each test article administration group were provided. The observations and examinations included clinical observations, body weight, food consumption, ophthalmology, urinalysis, hematology, blood chemistry, gross pathology, organ weights and histopathology.

There were no deaths due to administration of the test article. One male in the 1 mg/kg group died on day 138, but the cause of death was unknown. One male in the 100 mg/kg group died on day 8 from large granular lymphocytic leukemia. This finding was not related to treatment with PROJECT Y since it was not observed thereafter and it was unrelated to dose level. One male in the 100 mg/kg group was sacrificed moribund on day 164 and 1 female in the same group on day 141. The cause of moribundity was adenoma and malignant lymphoma in the anterior lobe cells in the pituitary, respectively. The malignant lymphoma was considered to be unrelated to treatment with PROJECT Y since it occurs spontaneously in Sprague Dawley rats, is observed in the background data, was seen in only one rat and the finding was not dose related.

In males and females at all dose levels, there were no test article-related effects in ophthalmology. There were no abnormalities during clinical observations, except for the small testes in males in the 10 mg/kg dose group and above detected upon palpation during the detailed clinical observations.

At ≥ 1 mg/kg, females showed a decrease in the number of corpora lutea associated with a decrease in ovary weight, and high values in body weight and food consumption. In addition, hypertrophy of interstitial gland cells was observed in the ovaries at 1 and 10 mg/kg, and increased squamous metaplasia of the glands in the uterus was noted at 1 mg/kg.

At ≥ 10 mg/kg, males did not show effects from administration of the test article during the blood chemistry examinations, but they showed small testes, epididymides, seminal vesicles and prostate, decreased testes, seminal vesicles and prostate weights, seminiferous tubular atrophy and interstitial cell atrophy in the testes, hypospermia, ductal atrophy and cell debris in the ductal lumen of the epididymides, glandular atrophy in the seminal vesicles and prostate, atrophy of the zona reticularis in the adrenals, atrophy of the mammary glands, a decrease in trabecular bone in the femur, and an increase in adipocytes in bone marrow in the femur and sternum. At these doses, females had high values in ALP, low calcium, total protein, albumin, globulin and A/G ratio values, small ovaries and uterus, low pituitary, adrenal and uterus weights, an increase in the number of atretic ovarian follicles and atrophy of interstitial gland cells in the ovaries, atrophy of the uterus, mucosal atrophy and mucosal cell infiltration in the vagina, atrophy of the zona reticularis in the adrenal glands, anterior lobe atrophy in the pituitary, atrophy of the mammary glands, a decrease in the trabecular bone in the femur, and an increase in adipocytes in bone marrow in the femur.

At ≥ 100 mg/kg, males showed low values in body weight and food consumption, decreased number of sperm in the urine sediments, low fibrinogen and inorganic phosphorus values, high creatinine values, decreased liver and kidney weights, and increased thymus weights.

Females had small adrenal glands associated with atrophy of zona reticularis and hypertrophy of zona glomerulosa, decreased trabecular bone in the sternum and an increase in adipocytes in bone marrow in the sternum.

At 1000 mg/kg, males and females showed retention of pale yellow material in the stomach and cecum, erosion and mucosal hemorrhage in the glandular stomach, and hyperplasia of the limiting ridge. In addition, males had low triglycerides values and high total cholesterol values, and females had high urinary volumes, high one-day excretion of sodium, potassium and chloride, low urinary osmotic pressure, and high fibrinogen and ALP isoenzymes

(bone-type alkaline phosphatase , liver-type alkaline phosphatase and intestinal tract-type alkaline phosphatase) values.

In each dose group, dose-dependent low serum testosterone concentrations were noted in males. The values were lower than the detection limit (0.15 ng/mL) at doses of ≥ 100 mg/kg.

The Cmax and AUC24 on day 1 and weeks 13 and 26 increased dose-dependently in both males and females. The Cmax and AUC24 showed consistent tendencies throughout the administration period. The Cmax and AUC24 in each dose group tended to be slightly higher in females than in males, except for AUC24 at 1000 mg/kg in weeks 13 and 26.

Among the changes described above, the decrease in the serum testosterone concentrations and the changes in the reproductive organs (except mucosal cell infiltration in the vagina) were thought to be due to the pharmacological effects of the test article, a GnRH receptor

antagonist, and thus were not considered to be toxicological findings. The changes observed in body weight, pituitary, thymus, femur, sternum, femoral bone marrow, sternal bone marrow and mammary gland were also thought to be related to the pharmacological effects of the test article. However, the decrease in trabecular bone in the sternum and femur was judged to be adverse since it is an effect which could be translated to humans.

The NOAEL was estimated to be 1 mg/kg per day.

### 1-Week Oral Dose Range-finding Study in Dogs (Exploratory)

Study Project Y-TX-0018 (not GLP compliant) was conducted to evaluate the potential toxicity of PROJECT Y following a daily oral administration in dogs for 1 week. PROJECT Y was orally administered once daily for 1 week to beagle dogs (1 animal/sex per group) at 0, 10, 30, 100 and 300 mg/kg per day. Effects on clinical signs, body weight, food consumption, electrocardiography, hematology, blood chemistry, and pathologies were evaluated.

Systemic exposure was also investigated.

Increase in ALP level was observed in the male at 30 mg/kg. At 100 mg/kg per day and higher, elevated ALP was observed in both sexes. At ≥ 100 mg/kg, vomitus and stool in which both contained test article, were also noted in both males and females.

Cmax and AUC24 values after the first administration were increased in a dose-proportional manner except for 300-mg/kg administration. There were no apparent differences in these values between 100 and 300 mg/kg administrations. After the seventh administration, these parameters showed the same tendency as the first administration.

The NOAEL under the conditions of this study was 10 mg/kg per day in the male and 30 mg/kg per day in the female.

### 4-Week Repeated Oral Dose Toxicity Study in Dogs

Study Project Y-TX-0006 (GLP compliant) was conducted to evaluate the potential toxicity of PROJECT Y following daily oral administration in beagle dogs for 4 weeks. This study was conducted by suspending the PROJECT Y solid dispersion in water for injection (vehicle). The reversibility of any effects was also assessed following an 8-week untreated recovery period.

PROJECT Y was orally administered once daily to beagle dogs (3/sex/group) for 4 weeks at 0, 3, 10, and 300 mg/kg per day. These doses are equivalent to 6, 20 and 600 mg/kg of PROJECT Y solid dispersion, respectively (PROJECT Y:TC-5 [hypromellose] = 1:1). The dosing formulations were prepared by suspending PROJECT Y in water for injection (vehicle). Control animals (3/sex) received the vehicle alone in a similar manner. The reversibility of any effects was assessed following an 8-week untreated recovery period for 3 animals/sex in the 300 mg/kg group. The observations and examinations included clinical signs, body weight, food consumption, ophthalmology, electrocardiography, hematology, blood chemistry, testosterone measurement, urinalysis, gross pathology, organ weight and histopathology.

Measurements for plasma concentrations of PROJECT Y were conducted to perform toxicokinetic analysis.

All animals survived the duration of the study.

Pharmacology related effects included atrophic changes in the genital organs in males and females and decreased testosterone concentrations in males at all doses of PROJECT Y.

Loose and muddy stools and vomitus were observed for a female at 10 mg/kg and males and females at 300 mg/kg. The clinical chemistry examination revealed increases in ALP, total cholesterol, triglycerides and phospholipids in both sexes in the 300-mg/kg group. Decreases in the red blood cell count, hemoglobin concentration and hematocrit were found in 1 female in the 300-mg/kg group. At this dose, also an increase in relative liver weight was noted in males.

One male in the 300-mg/kg group exhibited a distinctively higher exposure level than the other animals in the same group, especially on day 28 of dosing. This male exhibited various signs indicative of a deterioration of its general physical condition.

The Cmax and AUC24 values in both sexes in the 3, 10 and 300 mg/kg groups increased with increasing dose levels. During repeated dosing, the Cmax and AUC24 values were almost constant or slightly decreased. There was no appreciable gender difference.

The NOAEL was 3 mg/kg per day. The toxicological and pharmacological findings induced by PROJECT Y were recovered or tended to recover during the 8-week recovery period.

### 39-Week Repeated Oral Dose Toxicity Study in Dogs

Study Project Y-TX-0020 (GLP compliant) was conducted to evaluate the potential toxicity of PROJECT Y solid dispersion following daily oral administration in beagle dogs for 39 weeks. In addition, drug concentrations in plasma were determined to examine systemic exposure to PROJECT Y.

PROJECT Y solid dispersion was orally administered once daily for 39 weeks to beagle dogs (4/sex per group) at 0, 1, 3, 10 and 100 mg/kg per day. Control animals (4/sex) received the vehicle alone (water for injection) in a similar manner. The observations and examinations included clinical signs, body weight, food consumption, ophthalmology, electrocardiography, hematology, blood chemistry, testosterone measurement, urinalysis, gross pathology, organ weight, histopathology and toxicokinetic analysis.

No test article-related changes except for those concerning or relating to the pharmacological action of the test article were observed in any animal at 1, 3 or 10 mg/kg.

At 100 mg/kg, vomiting with or without test article-like material was noted in 1 male and

2 females at 100 mg/kg sporadically (30 to 36 times per animal) during the dosing period. In the blood chemistry, a high value for ALP was noted in both sexes in weeks 4 to 39 (except for males in week 4). Analysis of ALP isoenzymes from the plasma obtained on the day of necropsy revealed high values for total ALP and liver-type ALP isoenzyme in both sexes. In addition, high triglyceride values were noted in males in weeks 4 to 39. Relating to this change, an increase in absolute weight of the liver with larger size was noted in males.

As a pharmacological action of the test article on the genital organs, decreases in the absolute and/or relative weight of the testis, epididymis and prostate in males, and atrophy of the ovary in females were noted at ≥ 1 mg/kg. Decreases in the absolute and relative weights of

the ovary and those of the uterus with smaller size were noted in females at ≥ 3 mg/kg. Histopathological examination revealed atrophy of the prostate in males, and atrophy of the uterus and vagina in females at ≥ 3 mg/kg. Atrophy of the mammary gland was also noted in one female at 1 and 3 mg/kg. Smaller testis, epididymis and prostate size in males, and extinction of estrus hemorrhage and smaller ovary size in females were noted at 10 and

100 mg/kg. Histopathological examination revealed atrophy of the seminiferous tubules in the testis, atrophy of the interstitial cells in the testis, hypospermia in the epididymis, and atrophy of the ducts in the epididymis in males at 10 and 100 mg/kg. Cell debris in the ductal lumen in the epididymis was also noted in 1 male at 10 mg/kg.

In addition, decreased trabecular bone of the femur was noted in females at ≥ 3 mg/kg; however, it was a minimal change restricted in the trabecular bone, and considered to be a secondary effect of the test article on the ovary, that is, suppressive effect on estrogen secretion. Therefore, it was judged to be a minimal adverse effect that was secondarily related to the pharmacological action of the test article.

In association with the pharmacological changes mentioned above, decreases in plasma testosterone were noted in males at 1, 3 and 10 mg/kg mainly at 4 and 8 h after dosing, and in males at 100 mg/kg at 2 to 24 h after dosing. Contrarily, increases in the testosterone concentration at 24 h after dosing were noted in males at 1, 3 and 10 mg/kg.

The Cmax and AUC24 values increased almost dose-proportionally from 1 to 100 mg/kg. These values showed a tendency to be almost constant during the dosing period. No sexual differences were observed in the Cmax or AUC24 at any dose. The mean tmax values were

0.9 to 2.5 h, and almost constant during the dosing period.

The NOAEL was 10 mg/kg per day for males and 1 mg/kg per day for females.

### In Vitro Reverse Mutation Test

Study Project Y-TX-0001 (GLP compliant) was conducted to evaluate PROJECT Y for its potential to induce gene mutation in bacteria. A bacterial reverse mutation test was performed with

5 strains of bacteria (*Salmonella typhimurium* [TA98, TA100, TA1535, and TA1537] and *Escherichia coli* [WP2*uvrA*]), using the pre-incubation method with and without metabolic activation.

The dose-finding test and the main test were performed at the following dose levels: Dose-finding test:

* Without and with metabolic activation:

5, 15, 50, 150, 500, 1500, and 5000 µg/plate (all strains) Main test:

* Without metabolic activation:

78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate (all strains)

* With metabolic activation:

156, 313, 625, 1250, 2500, and 5000 µg/plate (all strains)

In comparison with the negative control, no 2-fold or greater increases in revertant colonies were observed in any test strain in both tests, without or with metabolic activation. It was concluded that, under the conditions of this study, PROJECT Y did not induce gene mutation in bacteria.

### In Vitro Chromosomal Aberration Test

Study Project Y-TX-0002 (GLP compliant) was conducted to evaluate PROJECT Y for its potential to induce chromosomal aberration in cultured mammalian cells. A chromosomal aberration test was performed with cultured mammalian cells (CHL/IU cells) using short-term treatments for 6 h with and without metabolic activation and continuous treatment for 24 h without metabolic activation. The dose levels were set at 100, 200, 300, 400, and 500 µg/mL in short-term treatment with and without metabolic activation, and at 50, 75, 100, 125, and 150 µg/mL in continuous treatment for 24 h, and the incidence of cells having structural and numerical chromosomal aberrations was investigated. A vehicle (DMSO) was selected as the negative control. A mitomycin C-treated group for treatment without metabolic activation and a benzo[a]pyrene-treated group for treatment with metabolic activation were selected as the positive control groups.

Significant increases in the incidence of cells having structural chromosomal aberrations were noted in the test article groups when compared with the negative control at 400 and

500 µg/mL, and 300, 400 and 500 µg /mL in short-term treatment with and without metabolic activation, respectively. No significant increases in the incidence of cells having numerical chromosomal aberrations were noted. The number of viable cells showed a dose-dependent decrease at tested concentrations.

It was concluded from these results that, under the conditions of this study, PROJECT Y induced chromosomal aberrations in CHL/IU cells in short-term treatment with and without metabolic activation at concentrations that decreased cell viability.

### Micronucleus Test in Rats

In Study Project Y-TX-0014 (GLP compliant), a micronucleus test was performed in order to assess the potential of PROJECT Y solid dispersion to induce micronuclei caused by chromosomal aberrations in vivo in rat erythroblasts. Systemic exposure to PROJECT Y was also investigated. PROJECT Y solid dispersion was administered orally once daily for 2 days (at an approximately 24-hour interval) to male and female Sprague Dawley rats (5/sex/group) at 0, 250, 500, 1000 and 2000 mg/kg per day. The incidence of micronucleated immature erythrocytes (MNIE%) and the ratio of immature erythrocytes (IE%) were investigated in femoral bone marrow at approximately 24 h after the final dosing. A vehicle (water for injection) was administered orally once daily for 2 days (at an approximately 24-hour interval) in the same manner. As a positive control, 20 mg/kg of cyclophosphamide monohydrate was administered orally once. Toxicokinetic analysis was conducted with satellite animals in the 2000-mg/kg group on the second administration.

No significant increase in MNIE% was noted in any test article group when compared with the negative control group. A significant decrease in IE% was noted at 2000 mg/kg per day in males and 500, 1000, and 2000 mg/kg per day in females when compared with the negative control group. No animals died, and no test article-related changes were observed in clinical signs after dosing. No suppression of body weight gain was noted. The Cmax and AUC24 of plasma PROJECT Y confirmed exposure to the test article.

It was concluded that PROJECT Y did not induce micronuclei caused by chromosomal aberrations in vivo in male and female rats when tested under the conditions of this study.

### Unscheduled DNA Synthesis Test in Rats

In Study Project Y-TX-0015 (GLP compliant) the genotoxic potential of PROJECT Y to induce DNA damage in vivo was assessed by measuring unscheduled DNA synthesis (UDS) in rat hepatocytes as an index.

PROJECT Y solid dispersion was examined for its ability to induce UDS in rat hepatocytes in order to evaluate the effects to induce DNA damage in vivo. PROJECT Y was orally administered once to 3 male Sprague Dawley rats/treatment group at 0, 500, 1000 and 2000 mg/kg. Hepatocytes were isolated at 2 or 16 h after the administration and UDS induction was examined using autoradiography. Specimens were prepared to calculate net grains and percentage of cells in repair.

The data obtained from animals treated with the test substance were comparable to those in the negative control groups. Based on these results, it was judged that the test substance was negative in the UDS test using rat livers.

It was concluded that PROJECT Y has no ability to induce DNA damage in vivo.

### Carcinogenicity

No longer-term studies have yet been conducted with PROJECT Y, but the genotoxicity data shows no reason for concern. Two- and 13-week oral dose range finding studies in mice (Project Y-TX-0022 [not GLP compliant] and Project Y-TX-0023 [GLP compliant]) and rats (Project Y-TX-0025 [not GLP compliant] and Project Y-TX-0026 [GLP compliant]) have been conducted. The data obtained are summarized in [End-of-Text Table 3.9].

### Reproductive and Developmental Toxicity

Fertility and early embryonic development studies have been conducted in both male and female rats. Embryofetal development studies have been conducted in pregnant female rats and rabbits. No studies have been conducted to assess pre and postnatal development or toxicity in juvenile animals. The results of the reproductive and development toxicity studies are summarized in [End-of-Text Table 3.10].

### Effects on Fertility and Early Embryonic Development to Implantation

Fertility and early embryonic development was assessed in male (Study Project Y-TX-0011) and female rats. Female rats were assessed with treatment in the pre-mating period and treatment in the early stages of pregnancy (Studies Project Y-TX-0012, Project Y-TX-0013 and Project Y-TX-0021).

An additional study during the pre-mating period was also conducted in female rats to further assess toxicokinetics (Study Project Y-TX-0016) [End-of-Text Table 3.11].

### Definitive Study in Male Rats

In Study Project Y-TX-0011 (GLP compliant) the potential adverse effects of PROJECT Y on mating, fertility and early embryonic development in rats was evaluated when administered orally to male rats from 4 weeks prior to mating, through the mating period until 1 day prior to necropsy.

PROJECT Y solid dispersion was orally administered once daily to 20 male Sprague Dawley rats/group at 0 (control), 0.2, 1 and 10 mg/kg per day from 28 days prior to mating with untreated females, through the mating period until 1 day prior to necropsy (63 to 66 days in total). An additional 20 males (recovery animals) received 10 mg/kg per day for 28 days and were allowed to recover for 8 weeks to evaluate the reversibility of any effects. PROJECT Y solid dispersion was suspended in water for injection and administered. On day 13 of gestation, pregnant females underwent Cesarean section and were necropsied.

In the male parental animals, no findings suggestive of treatment-related effects were evident in any treated group. With regard to the effects on early embryonic development, no treatment-related effects were evident on the number of corpora lutea, implantations or live embryos or preimplantation or postimplantation loss.

The NOAEL of PROJECT Y was 10 mg/kg per day for general toxicity and paternal reproductive function of males and early embryonic development.

### Exploratory Study in Female Rats (Treatment During Premating Period)

In Study Project Y-TX-0012 (not GLP compliant) potential adverse effects of PROJECT Y on mating, fertility and early embryonic development in rats was evaluated when administered orally to females for 2 weeks prior to mating and the mating period. The results of this study were used to select dose levels for the subsequent definitive study.

PROJECT Y solid dispersion was orally administered once daily to 6 female Sprague Dawley rats/group for 2 weeks prior to mating and during the mating period at 0 (control), 1, 10 and 1000 mg/kg per day. PROJECT Y solid dispersion was suspended in water for injection and administered. In the 1000 mg/kg group, dosing was discontinued on day 21 of dosing and the animals were maintained untreated for 3 weeks to evaluate the recovery of the estrous cycle. On day 13 of gestation, pregnant females underwent Cesarean section and were necropsied.

In the females, increased body weight gain and food consumption were noted in the 10- and 1000-mg/kg groups. Cessation of the estrous cycle was evident during the premating period in the 1000-mg/kg group and irregular estrous cycles were noted in the 1- and 10-mg/kg group. The copulation indices were decreased to 66.7% and 0% in the 10- and 1000-mg/kg groups, respectively. The fertility indices were decreased to 16.7% and 0% in the 1- and

10-mg/kg groups, respectively. In treated animals, only 1 female in the 1-mg/kg group became pregnant and the preimplantation loss (%) in this animal was high (75.0%). In this

group, decreases in the numbers of corpora lutea and implantations and an increase in the post implantation loss (%) were also evident.

The estrous cycle returned to normal promptly after the start of the recovery period in 2 animals in the 10-mg/kg group that failed to mate. The estrous cycle also returned to

normal in half of the animals in the 1000-mg/kg group after 2 weeks from the start of the recovery period.

Based on the results of this study, the following dose levels were considered to be appropriate for the subsequent definitive study: 10 mg/kg per day, at which clear effects on the copulation index and fertility index would be expected, as the high dose level; and 0.2 and

0.01 mg/kg per day as the mid and low dose levels, respectively, to evaluate the NOAEL of PROJECT Y.

### Definitive Study in Female Rats (Treatment During Premating period)

In Study (Project Y-TX-0013) (GLP compliant) any potential adverse effects of PROJECT Y on mating, fertility and early embryonic development in rats was evaluated when administered orally to females for 2 weeks prior to mating and the mating period. The reversibility of any effects was also assessed following a 4-week untreated recovery period. The toxicokinetic profile of PROJECT Y was also assessed.

PROJECT Y solid dispersion was administered orally to 20 female Sprague Dawley rats/group at 0 (control), 0.01, 0.05, 0.2 and 10 mg/kg per day for 2 weeks prior to mating and during the mating period with untreated males. Another 20 females received 10 mg/kg per day for 14 days and were allowed to recover for 4 weeks to evaluate the reversibility of any effects (recovery animals). PROJECT Y solid dispersion was suspended in water for injection and administered. On day 13 of gestation, pregnant females underwent Cesarean section and were necropsied.

No general toxicological effects were evident on physical condition, body weights, food consumption or gross pathology of female parental animals. Estrous cycles were prolonged and the copulation and fertility indices were decreased in the 10-mg/kg group. Decreases in the numbers of corpora lutea were evident in the 10-mg/kg group. The estrous cycle, mating and fertility indices and the number of corpora lutea tended to recover following the 4-week recovery period in the 10-mg/kg recovery animals.

With regard to the effects on embryo-fetal development, decreases in the numbers of implantations and live fetuses and increases in the pre and postimplantation losses (%) were evident in the 10-mg/kg group. The numbers of implantations and live fetuses and the pre and postimplantation losses (%) tended to recover following the 4-week recovery period in the 10-mg/kg recovery animals. At 0.2 mg/kg or less, there was no effect on reproductive function of female parental animals and for early embryonic development.

Plasma concentrations of PROJECT Y in the 0.01-mg/kg group were below the LLOQ on days 1 and 14 of dosing. The plasma PROJECT Y concentrations in the 0.05-mg/kg group after the first dosing were detected at only 2 time points; therefore, the toxicokinetic parameters were

not calculated. The Cmax and AUC24 values in the 0.05-mg/kg group after the last dosing were 2.42 ng/mL and 4.11 ng·h/mL, respectively.

The NOAELs were 10 mg/kg per day for general toxicity in female parental animals and

0.2 mg/kg per day for reproductive function of female parental animals and for early embryonic development.

### Additional Toxicokinetic Determination Study for Definitive Study in Female Rats

The NOAEL of PROJECT Y for reproductive function of female rats was 0.2 mg/kg in a “Study for Effects of PROJECT Y Solid Dispersion on Fertility and Early Embryonic Development to Implantation in Rats via Oral Administration – Female Dosing Study (Treatment in the

Pre-Mating Period) -” previously conducted by Ina Research Inc. (Study No. Project Y-TX-0013). However, since no toxicokinetic data were available for this dose level, an additional toxicokinetic analysis was conducted at 0.2 mg/kg under the same study conditions.

In this study (GLP compliant), PROJECT Y solid dispersion was orally administered once daily to 9 female Sprague Dawley rats at 0.2 mg/kg per day for 14 days and plasma PROJECT Y concentrations were determined to evaluate the systemic exposure of the animals to PROJECT Y.

All animals survived the duration of the study. No general toxicological effects of PROJECT Y were evident on physical condition or body weights of animals at 0.2 mg/kg as in the previous study.

Cmax and AUC24 values were 5.17 ng/mL and 16.62 ng·h/mL at initial dosing and 5.88 ng/mL and 16.00 ng·h/mL at final dosing, respectively.

The NOAELs of PROJECT Y were confirmed at 0.2 mg/kg per day for reproductive function of female parental animals and for early embryonic development.

### Definitive Study in Female Rats (Treatment During Early Stage of Pregnancy)

The purpose of Study (Project Y-TX-0021) (GLP compliant) was to evaluate any potential adverse effects of PROJECT Y on early embryonic development to implantation in rats when administered orally to female rats from Days 0 to 7 of gestation. The toxicokinetic profile of PROJECT Y was also assessed.

PROJECT Y solid dispersion was orally administered to 19 or 20 pregnant female Sprague Dawley rats/group at 0 (control), 0.05, 0.2, 1 and 10 mg/kg per day from days 0 to 7 of gestation. PROJECT Y solid dispersion was suspended in water for injection and administered.

Clinical observations and measurements of body weights and food consumption were conducted for the dams. On day 20 of gestation, the dams underwent Cesarean section and necropsy to determine the numbers of corpora lutea, implantations, live and dead fetuses; sex ratio, body weights, placental weights and external morphology of live fetuses.

Toxicokinetic analysis was conducted with satellite animals on day 0 and 7 of gestation.

All dams survived the duration of the study exhibiting no treatment-related effects on physical condition, body weight, food consumption or gross pathology. Suppressed body weight gains were noted in dams at 10 mg/kg during the later stages of gestation. However, this was due to growth retardation of the fetuses and it was considered that there were no effects of the test article on the body weight gain in dams.

Delayed implantation was noted at 10 mg/kg, but no treatment-related effects were evident on maintenance of pregnancy thereafter in any dam.

Marked decreases in fetal weights, decrease in placental weights and premature fetuses were noted at 10 mg/kg. No treatment-related effects were evident on embryo-fetal viability.

The Cmax and AUC24 values increased more than dose-proportionally up to 10 mg/kg on

days 0 and 7 of gestation. After repeated dosing, the values on day 7 of gestation were nearly comparable to those on day 0 of gestation.

The NOAEL of PROJECT Y was considered to be 1 mg/kg per day for both early embryo-fetal development and reproductive function of dams when treated during the early stage of pregnancy.

### Effects on Embryo-fetal Development

Embryofetal development was assessed in both the rat and rabbit.

### Exploratory Study in Rats

The purpose of Study (Project Y-TX-0007) (not GLP compliant) was to evaluate the potential adverse effects of PROJECT Y on pregnant animals and embryo-fetal development (embryo- fetal death, growth retardation and external malformations) when administered orally to pregnant rats on days 7 to 17 of gestation that correspond to the period from implantation to closure of the hard palate of rat fetuses.

The results of this study were used to select dose levels for the subsequent definitive study.

PROJECT Y solid dispersion was orally administered once daily to 5 or 6 pregnant Sprague Dawley rats/group at 0 (control), 1, 10, 100 and 1000 mg/kg per day on days 7 to17 of gestation that correspond to the period from implantation to closure of the hard palate of rat fetuses. PROJECT Y solid dispersion was suspended in water for injection. On day 20 of gestation, the dams underwent Cesarean section and were necropsied.

In dams, flushing of the ears and scant feces were observed in the 1000-mg/kg group. Suppressed body weight gain in the 10-, 100- and 1000-mg/kg groups and decreased food consumption in the 100 and 1000 mg/kg groups were noted.

In fetuses, increased postimplantation loss (%) was noted in the 10-, 100- and 1000-mg/kg groups and live fetuses could not be obtained in the 100- or 1000-mg/kg group (total litter loss). No external malformations were evident in any live fetus in the 10-mg/kg group.

Total litter loss was noted in all dams and live fetuses could not be obtained in the 100 and 1000 mg/kg groups. Increased post-implantation loss (65.1%) was noted in the 10 mg/kg

group. Therefore, 10 mg/kg was judged to be too high to obtain sufficient number of live fetuses to evaluate.

Based on the results of this study, the following dose levels were considered to be appropriate for the subsequent definitive study: 5 mg/kg per day as the high dose level since no effects on dams or post-implantation loss (%) were noted at 1 mg/kg per day; and 1 and 0.2 mg/kg per day as the mid and low dose levels, respectively, using a common ratio of 5.

### Definitive Study in Rats

The purpose of this Study(Project Y-TX-0008) (GLP compliant) was to evaluate the potential adverse effects of PROJECT Y on pregnant animals and embryo-fetal development

(embryo-fetal death, growth retardation and malformations) when administered orally to pregnant rats on days 7 to 17 of gestation that correspond to the period from implantation to closure of the hard palate of rat fetuses. The toxicokinetic profile of PROJECT Y was also assessed.

PROJECT Y solid dispersion was suspended in water for injection and orally administered once daily to 19 to 20 pregnant Sprague Dawley rats/group at 0 (control), 0.2, 1 and 5 mg/kg per day on days 7 to 17 of gestation that correspond to the period from implantation to closure of the hard palate of rat fetuses. On day 20 of gestation, the dams underwent Cesarean section and were necropsied.

In dams, no treatment-related effects were observed on physical condition, body weights, food consumption, gross pathology or maintenance of pregnancy of dams in any treated group.

In fetuses, no treatment-related effects were evident on the viability, growth, external, visceral or skeletal morphology or ossification of embryos/fetuses in any treated group.

The Cmax and AUC24 values on days 7 and 17 of gestation increased more than

dose-proportionally up to 5 mg/kg. After repeated dosing, these values increased as dosing progressed.

The NOAEL was 5 mg/kg per day for maternal general toxicity, maternal reproductive function and embryo-fetal development.

### Exploratory Study in Rabbits

The purpose of Study (Project Y-TX-0009) (not GLP compliant) was to evaluate the potential adverse effects of PROJECT Y on pregnant animals and embryo-fetal development

(embryo-fetal death, growth retardation, external and visceral malformations) when administered orally to pregnant rabbits on days 6 to 18 of gestation that correspond to the period from implantation to closure of the hard palate of rabbit fetuses. The toxicokinetic profile of PROJECT Y was also assessed. The results of this study were used to select dose levels for the subsequent definitive study.

PROJECT Y solid dispersion was orally administered once daily to 6 mated New Zealand White rabbits/group at 0 (control), 0.1, 1, 10 and 100 mg/kg per day on days 6 to 18 of gestation

that correspond to the period from implantation to closure of the hard palate of rabbit fetuses. PROJECT Y solid dispersion was suspended in water for injection and administered. On day 29 of gestation, the dams underwent Cesarean section and were necropsied.

In dams, findings suggestive of maternal toxicity were not evident in physical condition, body weights, food consumption or gross pathology of dams in any treated group.

Implantation was not evident in 1 animal in the 1-mg/kg group, 2 animals in the 10-mg/kg group or in any of the 6 animals in the 100-mg/kg group. Two of 4 pregnant animals at 10 mg/kg showed high preimplantation loss rates (42.9 % and 46.2 %).

In fetuses, no treatment-related effects were noted on postimplantation viability or development and there were no statistically significant differences in the incidence of external or visceral malformations.

The Cmax and AUC24 values on days 6 and 18 of gestation increased greater than

dose-proportionally up to 10 mg/kg as a whole. The Cmax and AUC24 values on day 18 of gestation were comparable to those on day 6 for all dose levels.

Based on the results of this study, 10 mg/kg was judged to be too high to obtain a sufficient number of live fetuses to evaluate embryo-fetal development. No adverse effect on the preimplantation loss was noted at 1 mg/kg. Therefore, it was considered to be appropriate to select 5 mg/kg per day as the high dose level and 1 and 0.2 mg/kg per day as the mid and low dose levels, respectively, using a common ratio of 5 for the subsequent definitive study.

### Definitive Study in Rabbits

The purpose of Study (Project Y-TX-0010) (GLP compliant) was to evaluate the potential adverse effects of PROJECT Y on pregnant animals and embryo-fetal development (embryo-fetal death, growth retardation and malformations) when administered orally to pregnant rabbits on days 6 to 18 of gestation that correspond to the period from implantation to closure of the hard palate of rabbit fetuses. The toxicokinetic profile of PROJECT Y was also assessed.

PROJECT Y solid dispersion was suspended in water for injection and orally administered once daily to 18 pregnant New Zealand White rabbits/group at 0 (control), 0.2, 1 and 5 mg/kg per day on days 6 to 18 of gestation that correspond to the period from implantation to closure of the hard palate of rabbit fetuses. On day 29 of gestation, the dams underwent Cesarean section and were necropsied.

In dams, no treatment-related effects were observed on physical condition, body weights, food consumption, gross pathology or maintenance of pregnancy of dams in any treated group.

In fetuses, no toxicologically significant findings were evident on the viability, growth, external, visceral or skeletal morphology or ossification of embryos/fetuses in any treated group.

The systemic exposure in this study increased more than dose-proportionally over the dose range of 0.2 to 5 mg/kg.

The NOAEL was 5 mg/kg per day for maternal general toxicity, maternal reproductive function and embryo-fetal development.

### Local Tolerance

No local tolerance studies with PROJECT Y have been completed.

### Other Toxicity Studies

**4.3.6.1 In Vitro Phototoxicity Test**

The results of the phototoxicity study are summarized in [End-of-Text Table 3.16].

Study (Project Y-TX-0030) (GLP compliant) was conducted to investigate the potential phototoxicity of PROJECT Y in cultured mammalian cells (Balb/c 3T3 cells).

The dose range-finding test was performed at 0.3, 1, 3, 10, 30, 100, 300, and 1000 µg/mL in the presence and absence of UV-A irradiation. The IC50 could not be determined from the results of the dose range-finding test in the presence or absence of irradiation because cytotoxicity was not observed up to the highest non-precipitating dose. The results at

100 µg/mL and greater were not evaluated because precipitation of the test article was observed in the treatment mixture at 100 µg/mL and greater in the presence and absence of irradiation. Therefore, the main test was performed at 0.469, 0.938, 1.88, 3.75, 7.5, 15, 30 and 60 µg/mL in the presence and absence of UV-A irradiation.

The result was assessed from the mean photo effect (MPE), because the IC50 could not be determined in either the presence or absence of irradiation. The MPE was calculated to be

−0.002, meeting the criterion for no phototoxicity (MPE < 0.1). Accordingly, PROJECT Y was judged to have no potential to induce phototoxicity.

It was concluded that PROJECT Y showed no potential to induce phototoxicity to cultured mammalian cells.

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

In the currently available information from the safety pharmacology and toxicity studies, pharmacological effect-related atrophic changes in gonads, and in the pituitary, adrenal and mammary glands, decreased trabecular bone in rats and dogs, effects on fertility in rats and embryo-mortality in rats and rabbits, elevation of ALP levels in rats (females) and dogs, and an increase of liver weight in dogs, changes in clinical pathology parameters (lipids, proteins and blood coagulation in rats and dogs, urinary excretion in rats), erosion and mucosal hemorrhage of the stomach in rats, vomiting and loose and muddy stool in dogs, and changes in blood pressure and heart rate in dogs were noted.

Pharmacological effect-related changes in gonads were observed in 4- and 26-week oral dose studies in rats and 4- and 39-week oral dose studies in dogs. PROJECT Y is a GnRH receptor antagonist and inhibits release of LH and FSH from the pituitary. In males, T production was inhibited through inhibition of the release of LH, therefore, atrophic changes in the male gonads are anticipated. In both rats and dogs, dose dependent decreases in plasma T levels

occurred. Reduced organ weight and microscopically atrophic changes were noted in male gonads (testes, epididymis, seminal vesicle and prostate), changes that either decreased in extent or resolved after the withdrawal periods. It is likely that decreases in body weight and erythrocyte parameters in male rats were changes secondary to the chemical castration.

Effects on the pituitary and adrenals are also considered to be secondary changes, as a part of the pituitary-adrenal-gonadal axis. In females, the inhibition of FSH and LH leads to decreases in both estrogen and progesterone production. The decreases of plasma FSH and LH levels contribute to ovarian disorders and decreases in estrogen and progesterone levels induce atrophic changes in the female gonads. Plasma estrogen and progesterone levels were not measured during the toxicology studies; however, they were likely to be decreased because of the observed decreased ovary and uterus organ weights and atrophic changes in the gonads (ovary, uterus and vagina). After the recovery period, these findings disappeared or tended to recover in the 4-week studies. It is likely that increased body weight in female rats was a change secondary to the chemical castration. In the 26-week study in rats and the 39-week study in dogs, decreases in trabecular bone in the femur and sternum were observed, although the changes noted in dogs was minimal. These changes were considered to be a secondary effect of decreased estrogen due to the pharmacology effect of the test article.

However, it was judged to be adverse since it is an effect which could be translated to humans.

In the dose range-finding study for the female fertility study, effects on the estrous cycle, copulation index and fertility index occurred. Only 1 female in the 1-mg/kg group became pregnant and the preimplantation loss in this animal was high. At 1 mg/kg, decreases in the numbers of corpora lutea and implantations and an increase in the post implantation loss were also evident, indicating PROJECT Y has effects on embryonic survival. This finding was considered due to the reduced FSH and LH levels and imbalance of progesterone and estrogen based on the pharmacological effects. The definitive female fertility study revealed that the NOAEL was 0.2 mg/kg for the female fertility. In the dose range-finding study for effects on embryonic and fetal development in rats, an increase in postimplantation loss was noted at ≥ 10 mg/kg and no live fetuses were obtained at 100 mg/kg. These events are also considered to result from an imbalance of progesterone and estrogen due to the pharmacological effect. In the definitive study in rats, these effects were not observed at

5 mg/kg. Rabbits showed similar findings.

In the dog telemetry study, an increase in heart rate and a decrease in blood pressure were observed at ≥ 100 mg/kg and 1000 mg/kg, respectively. It is considered that these parameters should be also monitored in clinical studies.

Gastro-intestinal adverse findings consisted of vomiting and loose and muddy stools in dogs and erosion and mucosal hemorrhage in the stomach in rats. In rats, solid material (study drug) was present in the GI-tract, which may physically irritate the gastric mucosa.

Reversible increases in ALP and liver weight were observed in dogs. An increase in ALP was also noted in female rats. Together with changes in clinical chemistry parameters (cholesterol, phospholipids, triglycerides, aPTT, partial thromboplastin time, total protein and

albumin), this may point to potential effects on the liver. Despite these findings, no treatment-related histopathological changes in the liver and no toxicologically significant increases in AST, alanine aminotransferase (ALT) and total bilirubin levels were observed in rats and dogs.

Findings of potential concern for clinical trials included loss of BMD, gastrointestinal problems such as emesis and abnormal stool and elevation of ALP levels. In addition, heart rate increase should be monitored during early clinical trials. Other than these toxicological findings, major pharmacological effect-related changes occurred in gonads; pharmacodynamic parameters (T, LH and FSH) should be measured in the clinic to determine the extent of the pharmacological effect [[Table 2](#_bookmark29)].

The general toxicological profile is mild in nature and most events are reversible after a recovery period. The exposures at the doses giving toxicologically relevant findings surpass the exposure at the pharmacologically effective dose [[Table 3](#_bookmark30)].

### Table 2 Summary of Potential Safety Concerns

|  |  |
| --- | --- |
| **Potential Safety Concern (from nonclinical studies)** | **Relevance to Human Usage** |
| **Repeat-dose toxicity**   * Atrophic changes and decreased organ weight in male and female gonads related to the pharmacological effect. * Decreased trabecular bone. * Effects on laboratory tests (lipid, protein, and coagulation parameters).   (See Studies Project Y-TX-0005, Project Y-TX-0019, Project Y-TX-0006 and Project Y-TX-0020.) | * Pharmacology related changes in male and female gonads. * Loss of BMD * Potential changes in lipid, protein, and coagulation parameters. |
| **Reproductive toxicity**  - In female rats, disturbance of estrous cycle, decrease in fertility indices at  1 mg/kg, related to the pharmacological effect.  (See Study Project Y-TX-0013.) | * Disturbance of estrous cycle in females. * Potential effects on male libido. |
| **Developmental toxicity**  - Increases in pre and post implantation losses, decreased fetal bodyweight and premature fetuses at 10 mg/kg, related to the pharmacological effect.  (See Studies Project Y-TX-0013, Project Y-TX-0021, Project Y-TX-0007, Project Y- TX-0008, Project Y-TX-0009 and Project Y-TX-0010.) | - Potential for inducing miscarriage or reduced embryonic implantation. |
| **Hepatotoxicity**   * Increased ALP level in rats and dogs (observed at ≥ 30 mg/kg) * Increased liver weight in dogs at 300 mg/kg. * No toxicologically significant increase in AST or ALT. (See Study Project Y-TX-0019 and Project Y-TX-0006.) | - Potential for causing cholestatic liver effects such as ALP increase. |
| **Cardiovascular toxicity**  - Increased heart rate at  100 mg/kg and decreased blood pressure at 1000 mg/kg.  (See Study Project Y-PT-0004.) | - Potential for causing Cmax-related heart rate increase. |
| **Gastrointestinal toxicity**   * Vomiting in dogs at  10 mg/kg. * Loose or muddy stool in dogs at  10 mg/kg. * Erosion and mucosal hemorrhage in the stomach in rats at 1000 mg/kg.   (See Study Project Y-TX-0006 and Project Y-TX-0019.) | - PROJECT Y may cause nausea or diarrhea. |

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMD: bone marrow density

### Table 3 Exposure Ratio Based on Human AUC and Animal AUC of PROJECT Y

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species/**  **Study Duration** | **Dose** | **Sex (M/F)** | **AUC24**  **at Last Dose (ng·h/mL)** | **Exposure Ratio Based on Anticipated Clinical Effective Exposure** |
| Rat/4-week po | 10 mg/kg (NOAEL) | M | 1523.36 | 3.3 |
| F | 2989.83 | 6.5 |
| 100 mg/kg (LOAEL) | M | 49758.44 | 109 |
| F | 71006.37 | 155 |
| Rat/26-week po | 1 mg/kg (NOAEL) | M | 106.16 | 0.2 |
| F | 169.49 | 0.4 |
| 10 mg/kg (LOAEL) | M | 2808.52 | 6.1 |
| F | 4029.81 | 8.8 |
| Dog/4-week po | 3 mg/kg  (NOAEL) | M | 3215.15 | 7.0 |
| F | 2421.22 | 5.3 |
| 10 mg/kg (NOAEL) | M | 15102.33 | 33 |
| F | 11622.02 | 25 |
| Dog/39-week po | 10 mg/kg (NOAEL)  1 mg/kg (NOAEL) | M | 23003.52 | 50 |
| F | 1488.50 | 3.3 |
| 100 mg/kg (LOAEL)  10 mg/kg (LOAEL) | M | 146511.8 | 320 |
| F | 26114.42 | 57 |
| Anticipated clinical  effective exposure | 15 mg qd | F | 458† | NA |

† AUCtau at 15 mg qd in healthy female subjects (data from Project Y-CL-0001)

LOAEL: lowest observed adverse effect level; NA: not applicable; NOAEL: no observed adverse effect level. Source: Study Project Y-TX-0005, Project Y-TX-0019, Project Y-TX-0006 and Project Y-TX-0020.