* + 1. **Safety Pharmacology**

Potential effects of PROJECT 7 on hERG current and APD in vitro, and central nerve system in rats and cardiovascular and respiratory systems in dogs in vivo were examined. Results are summarized in [Table 5 - 3](#_bookmark24), and toxicokinetic data in [Table 5 - 4](#_bookmark25).

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

The effects of PROJECT 7 on the hERG current were studied in human ether-a-go-go-related gene transfected HEK 293 cells.

PROJECT 7 significantly suppressed the hERG current by 11.9% (compensated value) at 3x10- 5mol/L, but PROJECT 7 did not affect it at 3x10-6 mol/L.

* + - 1. ***In vitro* Effects on Action Potential Duration**

The effects of PROJECT 7 on action potential parameters in isolated guinea pig papillary muscle specimens were investigated using glass microelectrode techniques.

PROJECT 7 shortened APD30 by 9.4% at 3 x 10-5 mol/L. PROJECT 7 also shortened APD90 by 2.1% at 3 x 10-6 mol/L and 7.2% at 3 x 10-5 mol/L. However, no prolongation potential was indicated at any concentration. There was no effect on APD30-90, RMP, APA, or dV/dt max at 3 x 10-5 mol/L.

* + - 1. ***In vivo* Effects on Central Nervous System in Rats**

Six female rats per group received a single oral dose of PROJECT 7 solid dispersion (PROJECT 7:TC-5E (hydroxypropylmethylcellulose 2910) = 1:2) at dose levels of 0, 30, 100, and 600 mg/kg as PROJECT 7. The observation parameters included the general activity and behavior by modifying Irwin’s method.

PROJECT 7 had no effects on central nervous system at up to 600 mg/kg.

* + - 1. ***In vivo* Effects on Cardiovascular and Respiratory System in Dogs.**

Four male dogs received a series of single oral dose of PROJECT 7 solid dispersion at dose levels of 0, 10, 50, and 300 mg/kg as PROJECT 7, with the Latin-square design at an interval of 7 days.

PROJECT 7 did not affect the body temperature, blood pressure, heart rate, electrocardiogram, respiration rate, blood gases, or blood-electrolyte concentrations at doses of 10, 50, or 300 mg/kg.

As for the general activity and behavior, at 50 mg/kg, loose stool was observed in 1 animal. At 300 mg/kg, vomiting was observed in 3 animals; loose stool was observed in 1 animal, and diarrhea was observed in another animal.

These results indicate that PROJECT 7 solid dispersion has no effect on the cardiovascular and respiratory systems at doses up to 300 mg/kg as PROJECT 7.

# Table 5 - 3 Summary of Safety Pharmacology Studies of PROJECT 7

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species, Strain (M/F);**  **Test Model** | **Route** | **Dose** | **Endpoints** | **Major findings** | | | | **Study No. [GLP/Non- GLP]** |
| hERG  transfected HEK293  cells, N=5 | *In vitro* | 3x10-7,  3x10-6,  3x10-5  mol/L | Inhibition of hERG current | hERG current was suppressed by 11.9%  at 3x10-5mol/L  compensated inhibition rate 3x10-7 5.1%  3x10-6 4.6%  3x10-5 11.9%\* 1) | | | | Project 7-PT-0001  [GLP] |
| Guinea pig, Hartley, (M),  Isolated papillary muscle, N=5 | *In vitro* | 3x10-7,  3x10-6,  3x10-5  mol/L | Inhibition of action potential duration (APD);  APD30, APD90, APD30-90, RMP,  APA, dV/dt max | No effect on RMP, APA, dV/dt APD30 and APD90 were shortened, but  APD30-90 was not affected. | | | | Project 7-PT-0002 [GLP] |
|  | APD30 | APD90 | APD30-90 |
| 3x10-7 | 98.5 | 99.4 | 101.6 |
| 3x10-6 | 98.0 | 97.9\*2) | 97.6 |
| 3x10-5 | 90.6\*\*2) | 92.8\*\*\*2) | 98.7 |
| Rat, SD, (M) N=6,  Oral | Oral gavage | 0,  30,  100,  600  mg/kg | Abnormalities of; Mood,  Motor activity, Behavior, Motor coordination,  Central excitation | No effect | | | | Project 7-PT-0003 [GLP] |
| Dog, Beagle, (M), N=4,  Oral | Oral gavage | 0,  10,  50,  300  mg/kg | General activity and Behavior, Body temperature, Blood pressure, Heart rate, Electrocardiogram, Respiration rate Blood gas  Blood-electrolyte conc. | No effects on cardiovascular and respiratory systems.  General condition:  50 mg/kg: loose stool  300 mg/kg: vomiting, loose stool, diarrhea | | | | Project 7-PT-0004 [GLP] |

1. Significant difference at P0.05, PROJECT 7 vs vehicle control, Dunnett’s test.
2. \* P0.05, \*\* P0.01, \*\*\*PP0.001, compared with vehicle (Student’s t-test)

# Table 5 - 4 Summary of Toxicokinetic Data from Dogs in Safety Pharmacology Studies of PROJECT 7

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species, Strain** | **Gender (M/F)** | **No.** | **Route** | **Dose (mg/kg)** | **Cmax (ng/ml)** | **AUC**  **(ng.hr/ml)** | **Study No. [**GLP**/Non-**  **GLP]** |
| Dog Beagle | M | 4 | Oral gavage | 10 | 720 | 1624 | Project 7-PT-  0004 [GLP] |
| 50 | 5690 | 13632 |
| 300 | 25900 | 120503 |

* 1. **Toxicology**

Toxicity studies of PROJECT 7 performed to date are listed in [Table 5 - 10](#_bookmark38). Single dose, 1-week preliminary oral dose and 4-week oral dose with a 4-week recovery period toxicity studies have been conducted in rats and dogs. The animal species chosen for toxicology studies were based on the findings that both rats and dogs had the similar metabolic profile as humans *in vitro*. In addition, PROJECT 7 showed inhibitory activity against both rat and dog GnRH receptors. Dog was selected because dog enables higher systemic exposure to PROJECT 7 than monkeys. As for reproductive toxicity, maternal toxicity of PROJECT 7 and the effects on embryo-fetal development have been conducted in rats and rabbits. Fertility studies have been conducted separately for male and female rats. Genotoxicity studies have also been performed in *in vitro* and *in vivo* studies.

All of definitive studies were conducted in compliance with GLP and in accordance with relevant guidelines including those of ICH. Exploratory dose-range finding studies of repeated dose toxicity and reproductive toxicity were conducted under non-GLP.

PROJECT 7 solid dispersion (PROJECT 7:TC-5E (hydroxypropylmethylcellulose 2910) = 1:2) was used for *in vivo* studies.

# 

# Table 5 - 10 Summary of Nonclinical Safety Studies of *PROJECT 7*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Species, Strain** | **No. of**  **Animals/ Sex/Dose** | **Route** | **Duration**  **of Dosing** | **Doses (mg/kga)** | **Study No**  **[GLP/ Non-GLP]** |
| Single dose | Rat, SD | 5M, 5F | Oral,  gavage | Single | 0, 600, 1200b | Project 7-TX-0003  [GLP] |
| Dog, beagle | 1M, 1F | Oral,  gavage | Single | 300, 600c | Project 7-TX-0004  [GLP] |
| Repeat dose | Rat, SD | 5M, 5F | Oral,  gavage | 1 week (DRF) | 0, 30, 100, 300 | Project 7-TX-0029  [Non-GLP] |
| Rat, SD | 10M, 10F  (recovery 5M, 5F) | Oral, gavage | 4 weeks with 4-week  recovery | 0, 1, 30b), 100 a),  600 | Project 7-TX-0005 [GLP] |
| Dog, beagle | 1M, 1F | Oral,  gavage | 1 week (DRF) | 0, 30, 100, 300 | Project 7-TX-0030  [Non-GLP] |
| Dog, beagle | 3M, 3F  (recovery 3M, 3F) | Oral, gavage | 4 weeks with  4-week recovery | 0, 10 , 50, 300 | Project 7-TX-0006 [GLP] |
| Reverse mutation test | *S.*  *typhimurium*  and *E. coli* | - | *In vitro* | - | 6h (S9- and S9+),  24h (S9-); TA100, TA1535, TA1537:  39.1-2500 g/plate WP2uvr, TA98:  78.1-5000 g/plate | Project 7-TX-0001 [GLP] |
| Chromosomal aberration test | CHL/IU cells | - | *In vitro* | - | 6h (S9-); 15 - 270  g/mL  6h (S9+); 210 - 270  g/mL  24h (S9-); 15 - 90  g/mL | Project 7-TX-0002 [GLP] |
| Micronucleus  test | Mouse, ICR | 5M, 5F | Oral,  gavage | Single | 0, 300, 600, 1200b | Project 7-TX-0014  [GLP] |
| Unscheduled DNA  synthesis test | Rat, SD | 4M, 4F | Oral, gavage | Twice in a 2h interval | 0, 300, 600, 1200b | Project 7-TX-0015 [GLP] |

a For repeat-dose toxicity and reproductive toxicity, the NOAEL is underlined

b The animals were dosed with 30 mg/mL of PROJECT 7 twice at a volume of 20 mL/kg. The 2nd dose was conducted 2 hours after the 1st dose.

c The animals were dosed with 30 mg/mL of PROJECT 7 twice at a volume of 10 mL/kg. The 2nd dose was conducted 4 hours after the 1st dose.

a) NOAEL for male, b) NOAEL for female

# Table 5 - 11 Summary of Nonclinical Safety Studies of *PROJECT 7*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Species, Strain** | **No. of**  **Animals/ Sex/Dose** | **Route** | **Duration**  **of Dosing** | **Doses (mg/kga)** | **Study No**  **[GLP/ Non-GLP]** |
| Male fertility | Rat, SD | 20M | Oral,  gavage | See foot note d | 0, 1, 30, 100c),  600d) | Project 7-TX-0026  [GLP] |
| Female fertility (estrous cycle), DRF | Rat, SD | 6F | Oral, gavage | 14 days prior to mating with 2-week recovery  (DRF) | 0, 3, 100, 600 | Project 7-TX-0011 [Non-GLP] |
| Female fertility (ovulation),  DRF | Rat, SD | 4F | Oral, gavage | Single (DRF) | 0, 3, 100, 600 | Project 7-TX-0028 [Non-GLP] |
| Female fertility | Rat, SD | 20F | Oral,  gavage | See foot note e | 0, 1e), 30, 600f) | Project 7-TX-0012  [GLP] |
| Rat, SD | 20F | Oral,  gavage | Days 0-7 of  gestation | 0, 1, 30e), 100f), 600 | Project 7-TX-0013  [GLP] |
| Embryo-fetal development, DRF | Rat, SD | 8F | Oral,  gavage | Days 7-17 of  gestation | 0, 3, 30, 100, 600 | Project 7-TX-0007  [Non-GLP] |
| Rabbit, NZW | 6F | Oral,  gavage | Days 6-18 of  gestation | 0, 3, 30, 100, 300 | Project 7-TX-0008  [Non-GLP] |
| Embryo-fetal development | Rat, SD | 19-20F | Oral,  gavage | Days 7-17 of  gestation | 0, 1, 30g), 100f), 600 | Project 7-TX-0009  [GLP] |
| Rabbit, NZW | 17 or 19F | Oral,  gavage | Days 6-18 of  gestation | 0, 1, 3f), 30g) | Project 7-TX-0010  [GLP] |

a For repeat-dose toxicity and reproductive toxicity, the NOAEL is underlined

d The male animals were dosed 14 days prior to mating and throughout mating period. In the 600 mg/kg group, 14 days dosing period with 4-week recovery period was also set prior to mating.

e The female animals were dosed 14 days prior to mating and during the mating period. In the 600 mg/kg group, 14 day dosing and 6- week recovery period was also set prior to mating.

1. NOAEL for paternal general toxicity
2. NOAEL for paternal reproductive function and for embryonic development
3. NOAEL for maternal reproductive function and for early embryonic development
4. NOAEL for maternal toxicity
5. NOAEL for maternal reproductive function and embryo-fetal development
   * 1. **Single Dose Studies**

Single oral dose toxicity was examined in rats and dogs, and the results are summarized in [Table 5 - 12](#_bookmark41).

* + - 1. **Single Oral Dose Toxicity Study in Rats**

PROJECT 7 was dosed orally to SD rats at doses of 0, 600 and 1200 mg/kg as PROJECT 7. The high dose was achieved by duplicate dosing with a 2-hour interval of 600 mg/20 mL/kg. It was based on the maximum feasible concentration and the maximum volume that could be given to the animals. No animals died. Reddish brown urine was noted for males and females in the 1200 mg/kg group during 1 and 3 days after dosing. Transiently decreased food consumption was noted in the 600 and 1200 mg/kg groups on 1 day after dosing. No abnormalities were noted in body weight or gross pathology at up to 1200 mg/kg.

* + - 1. **Single Oral Dose Toxicity Study in Dogs**

PROJECT 7 was dosed orally to beagle dogs at doses of 300 and 600 mg/kg as PROJECT 7. The high dose was achieved by duplicate dosing with a 4-hour interval of 300 mg/20 mL/kg. It was based on the maximum feasible concentration and the maximum volume that could be given to the animals. No animals died. Vomiting and decreased food consumption were noted at 300 mg/kg or more, but no treatment-related effects were noted in hematology, clinical chemistry, gross pathology or histopathology at up to 600 mg/kg.

# Table 5 - 12 Summary of Single Dose Toxicity Studies of PROJECT 7

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Study** | **Species, Strain Dosing**  **details** | **Sex/ No./ dose**  **group** | **Doses (mg/kg)** | **Death** | **Major findings** |
| Project 7  -TX- 0003 | Single dose | Rat, SD,  Oral gavage | 5M, 5F | 0,  600,  1200a | None | Approximate lethal dose: greater than 1200 mg/kg  600 mg/kg: transiently decreased food consumption.  1200 mg/kg: reddish brown urine and transiently decreased food consumption |
| Project 7  -TX- 0004 | Single dose | Dog, Beagle, Oral gavage | 1M, 1F | 300,  600a | None | Approximate lethal dose: greater than 600  mg/kg  300 mg/kg: vomiting and decreased food consumption 4 hours after dosing  600 mg/kg: Vomiting immediate after dose and scant feces and loose stool 1 day after dosing |

a These doses were achieved by duplicate dosing

* + 1. **Repeated Dose Studies**

Exploratory 1-week repeated oral dose-range finding studies and definitive 4-week oral dose toxicity studies of PROJECT 7 with a 4-week recovery period were conducted in rats and dogs. Since the dose levels in the definitive studies covers those in the exploratory studies, the results of exploratory studies are shown only [in Table 5 - 13](#_bookmark43).

* + - 1. **4-week oral dose toxicity study in rats with a 4-week recovery period.**

PROJECT 7 was administered orally to rats at dose levels of 1, 30, 100 and 600 mg/kg once daily for 4 weeks. The highest dose level of 600 mg/kg was set as the maximum dose that can be administered to rats repeatedly. Clinical chemistry evaluations revealed increases in total cholesterol, phospholipids and calcium in females at 100 mg/kg and in males and females at 600 mg/kg. Increases in total protein and albumin were noted in females at 100 mg/kg or more. Decreased ovary weights were noted in females at 100 mg/kg or more. Decreased seminal vesicles weight were seen in males at 600 mg/kg. Increases in liver weight without related histopathological lesions were noted in females at 100 mg/kg and in both sexes at 600 mg/kg. Histopathology revealed cystic follicles and clearly decreased corpora lutea in females at 30 mg/kg or more. Ovarian findings are considered to be due to the major pharmacological effect of PROJECT 7. The reversibility of these changes was confirmed following a 4-week untreated period. No treatment-related effects were noted in clinical signs, body weight, food consumption, ophthalmology, hematology, urinalysis or gross pathology. The NOAELs were 100 mg/kg for males and 30 mg/kg for females.

* + - 1. **4-week oral dose toxicity study in dogs with a 4-week recovery period.**

PROJECT 7 was administered orally to beagle dogs for 4 weeks at dose level of 10, 50 and 300 mg/kg. Since the maximum practicable concentration of the preparation is 30 mg/mL, the maximum dose level for a repeated administration to dogs was set at 300 mg/10mL/kg. All animals survived the duration of the study. Loose stools were observed in a female at 50 mg/kg and males and females at the 300 mg/kg at a high frequency. Muddy or watery stools were also observed at 300 mg/kg. In addition, vomitus was noted in males and females at 300 mg/kg. These findings were suggestive of effects of PROJECT 7 on the digestive tract. Slight body weight loss and slightly decreased food consumption were noted in males at 300 mg/kg. Hematology evaluations revealed decreases in red blood cells count in males and females and decreases in hemoglobin concentrations and hematocrit in females at 300 mg/kg. All of these changes were no longer evident following a 4-week untreated recovery period.

No treatment-related effects were noted in electrocardiography, ophthalmology, clinical chemistry, urinalysis, gross pathology or histopathology. The NOAEL was 10 mg/kg.

# Table 5 - 13 Repeat Dose Toxicity Studies of PROJECT 7

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain Dosing**  **details** | **Sex/ No./ dose**  **group** | **Doses (mg/kg)** | **Death** | **Major findings** | **Study No.** |
| 1-week oral dose | Rat, SD,  Oral gavage | 5M, 5F | 0,  30,  100,  300 | None | Findings of pharmacological action were noted only in female in reproductive organs and adrenal.  30 mg/kg; corpora lutea , persistent of follicular cyst, estrous cycle was out of sync for the uterus and vagina.  100 mg/kg; luteal cyst in ovaries. 300 mg/kg: ovary weight , adrenal changes associated with compensatory steroid production; hypertrophy of the cells in the zona  reticularis and reduced number of lipid  droplets in the cells of the zona reticularis and fasciculata. | Project 7  -TX- 0029 |
| 4-week oral dose with a 4- week recovery | Rat, SD,  Oral gavage | 10M,  10F  (recovery 5M, 5F) | 0,  1,  30 a)  100b)  600 |  | Pharmacology related:  30 mg/kg: cystic follicles, number of corpora lutea,  100 mg/kg: ovary weight  600 mg/kg: seminal vesicle weight Liver:  100 mg/kg: liver weight (F) 600 mg/g: liver weight (M) Other:  100 mg/kg: total cholesterol, phospholipids, calcium, total protein, albumin(F)  600 mg/kg: total cholesterol,  phospholipids, calcium (M), | Project 7  -TX- 005 |
| 1-week oral dose | Dog, beagle | 1M, 1F | 0,  30,  100,  300 | None | No findings in clinical pathology or histopathology  30 mg/kg: salivation, drug-containing stool (F).  100 mg/kg: drug-containing vomiting (M, F),  300 mg/kg: food consumption and body  weight  (F). | Project 7  -TX- 0030 |
| 4-week oral dose with a 4- week recovery | Dog, Beagle, Oral gavage | 3M, 3F  (recovery 3M, 3F) | 0,  10,  50,  300 |  | GI tract:  50 mg/kg: loose stool (F)  300 mg/kg: loose stool (M), muddy/watery stool (M, F), vomitus (M, F)  Liver:  300 mg/kg: liver weight (M, F), 300 mg/kg: red blood cell (M, F), hemoglobin, hematocrit (F) Other:  300 mg/kg: salivation | Project 7  -TX- 0006 |

M: male, F: female, :increase, :decrease

NOAELs are shown with underline.

a): NOAEL for females, b): NOAEL for males

* + 1. **Carcinogenicity**

No studies have been conducted with PROJECT 7.

* + 1. **Special Studies**

No studies have been conducted with PROJECT 7.

* + 1. **Reproductive Toxicity**

An overview of reproductive toxicology studies is provided in [Table 5 - 14](#_bookmark47) and [Table 5 - 15.](#_bookmark48) The maternal toxicity of PROJECT 7 and its effects on embryo-fetal development were evaluated in preliminary (non-GLP dose range finding) and definitive studies in rats and rabbits. Since PROJECT 7 was expected to affect the reproductive system through the major pharmacological effect, the effects on fertility were evaluated separately for the male and female rats.

* + - 1. **Effects on Embryo-Fetal Development in Rats**

In the preliminary study, only decreased food consumption was evident at the 600 mg/kg/day in dams. However, 600 mg/kg is considered to be the maximum feasible dose. Therefore, the dose levels were set at 0, 1, 30, 100 and 600 mg/kg/day in the definitive study.

In the definitive study, dams showed transiently decreased food consumption at 600 mg/kg in the early stage of dosing. At the Caesarean sectioning, statistically significant increases in the pre-implantation loss, probably due to the major pharmacological action of the test substance, were noted in the 100 and 600 mg/kg groups as compared to the control group. No abortion, premature delivery or total litter loss occurred in any group and pregnancy was maintained in all dams. In fetuses, decreased fetal weights were noted in the 600 mg/kg group and delayed ossification was noted in the 100 and 600 mg/kg groups. No treatment-related effects were noted on embryo-fetal viability or morphology.

PROJECT 7 did not show teratological findings in this study. The NOAELs of PROJECT 7 were considered to be 100 mg/kg/day for maternal general toxicity, 30 mg/kg/day for reproductive function of dams and for embryo-fetal development.

* + - 1. **Effects on Embryo-Fetal Development in Rabbits**

Since a sufficient number of pregnant animal could not be obtained at 100 mg/kg/day or more in the preliminary study, the dose levels were set at 0, 1, 3 and 30 mg/kg/day in the definitive study. The increases in the non-pregnant rabbits in the preliminary study were considered to be due to implantation disturbance based on the inherent pharmacological action of PROJECT 7.

In the definitive study, dams showed a transient decrease in food consumption at 30 mg/kg. However, no other toxic effects were evident. No abortion, premature delivery or total litter loss occurred in any treated group, indicating successful maintenance of pregnancy in all dams in all treated groups. In fetuses, no treatment-related effects were evident on the viability, growth or morphogenesis of embryos/fetuses in any treated group.

PROJECT 7 did not show teratological findings in this study. The NOAELs of PROJECT 7 were considered to be 3 mg/kg/day for maternal general toxicity and 30 mg/kg/day for maternal reproductive function and for embryo-fetal development.

# Table 5 - 14 Reproductive Study: Summary of Effects on Embryo-Fetal Development

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain Dosing**  **details** | **Sex/ No./ dose**  **group** | **Doses (mg/kg)** | **Death** | **Major findings** | **Study No.** |
| Embryo–fetal development (DRF) | Rat, SD,  Oral, gavage | 8F | 0,  3,  30,  100,  600 | None | F0 dam:  600 mg/kg: food consumption ↓ F1 fetus:  No change | Project 7- TX-0007 |
| Embryo–fetal development (Definitive) | Rat, SD,  Oral, gavage | 19F  20F  20F  20F  20F | 0,  1,  30a),  100b),  600 | None | F0 dam:  ≥100 mg/kg: pre-implantation loss ↑  600 mg/kg: food consumption ↓ F1 fetus:  ≥100 mg/kg: delayed ossification ↑  600 mg/kg: body weight ↓ | Project 7- TX-0009 |
| Embryo–fetal development (DRF) | Rabbit, SD,  Oral, gavage | 6F | 0,  3,  30,  100,  300 | None | F0 dam:  100 mg/kg: 4/6 animals were not pregnant  300 mg/kg: 6/6 animals were not pregnant  ≥3 mg/kg: the percentage of pre-implantation loss ↑  F1 fetus:  30 mg/kg: fetuses with thymic remnant in neck ↑ | Project 7- TX-0008 |
| Embryo–fetal development (Definitive) | Rabbit, SD,  Oral, gavage | 19F  17F  19F  17F | 0,  1,  3 a),  30 b) | None | F0 dam:  30 mg/kg: food consumption ↓    F1 fetus:  No change | Project 7- TX-0010 |

↓: decrease, ↑: increase, F: female NOAELs are showed with underline

1. : NOAEL for maternal general toxicity
2. : NOAEL for maternal reproductive function and for embryo-fetal development
   * + 1. **Effects on Fertility in Male Rats**

PROJECT 7 was administered orally to male rats at dose levels of 0, 1, 30, 100 and 600 mg/kg/day from 2 weeks prior to mating, throughout the mating period until 1 day prior to necropsy. A transiently decreased food consumption was noted shortly after initiation of dosing at 600 mg/kg. However, changes suggestive of treatment-related effects were not evident in any treated group in clinical observations, body weights, gross pathology, weights of male genital organs, sperm evaluations, mating, fertility or pre-coital period in days. For early embryonic development, no treatment-related effects were evident on the number of corpora lutea, implantations or live embryos or pre- or post-implantation loss. The NOAELs of PROJECT 7 were considered to be 100 mg/kg/day for general toxicity in males and

600 mg/kg/day for reproductive function of males and for early embryonic development.

* + - 1. **Effects on Fertility in Female Rats**

Two kind of preliminary studies were conducted for effects on fertility of female rats. One was for the effect on the estrus cycles and its recovery, and the other was for the effect on the ovulation.

* + - * 1. **Exploratory Study for Effects on Estrus Cycle in Female Rats**

This study was designed to evaluate the effects of PROJECT 7 on estrus cycle when administered orally mature female rats at dose levels of 0, 3, 100 and 600 mg/kg/day for 14 days. The recovery groups were provided for the 100 and 600 mg/kg groups to assess the reversibility of abnormal estrus cycle by 14 days recovery period.

No abnormal sign was observed in any treatment groups. An increase in body weight gain and food consumption were observed at 600 mg/kg. An alteration of estrus cycle was not detected in the 3 mg/kg group. All animals in the 100 mg/kg group showed a persistence of the proestrus or estrous stages after the first estrous stage. Five of 6 animals in the 600 mg/kg group exhibited prolongation of diestrus phase in the early stage of administration period subsequently showed persistence of the metestrus phase. In another animal in this group, the estrus and diestrus cycle were persistent from the early stage of the administration period. In the recovery period, estrus and proestrus phase continued for a few days in all animals in the 100 and 600 mg/kg groups. After then, these abnormal estrus cycles showed tendency to recover.

* + - * 1. **Exploratory Study for Effects on Ovulation in Female Rats**

This study was designed to evaluate the effects of PROJECT 7 on ovulation when administered once orally to female rats at dose levels of 0, 3, 100 and 600 mg/kg/day in the morning on the day of proestrus. In the control group, all animals ovulated and the mean number ovulation was 11.8. In the 3 mg/kg group, all animals ovulated and the mean number. of ovulation was

10.0. There was no significant difference between this and the control group. However, 2 animals in the 3 mg/kg group showed unilateral ovulation. No ovulation was observed in animals in the 100 and 600 mg/kg group.

From these results, it was confirmed that PROJECT 7 completely inhibited the ovulation in rats at 100 mg/kg or more. It was suggested there is a possibility that 3 mg/kg of PROJECT 7 also possessed a potential to inhibit ovulation. Therefore, it was considered that the dosing periods of future planed “study for effects of PROJECT 7 on fertility and early embryonic development to implantation in female rats” should be divided into the premating period and the early stage of pregnancy to evaluate a effect on an early embryonic development. The setting of the less than 3 mg/kg was recommended to ensure no effect level for ovulation.

Based on the results from above 2 preliminary studies, following definitive studies for effects on fertility in female rats were examined dividing dosing period into premating period and the early stage of pregnancy, and 6-week recovery period was set in the study administered in the premating period.

* + - * 1. **Effects on Fertility in Female Rats (Treatment in the pre-mating period)**

PROJECT 7 was administered orally to female rats at dose levels of 0, 1, 30 and 600 mg/kg/day for 2 weeks prior to mating and during the mating period The recovery groups were provided for the 600 mg/kg group to assess the reversibility of abnormal estrus cycle by 6-week recovery period. All female parental animals survived the duration of the study. No general toxicological effects of PROJECT 7 were evident on general condition, body weights, food consumption or necropsy of female parental animals. Estrous cycles were prolonged in the 30 and 600 mg/kg groups and the mating index was decreased in the 600 mg/kg group. The fertility indices were decreased in the 30 and 600 mg/kg groups and no pregnant animals were obtained in the 600 mg/kg group. These changes were considered to be due to the inherent pharmacological action of PROJECT 7 (FSH and LH secretion inhibitory action).

There were no differences in the number of corpora lutea in the pregnant females between the control and the 1 or 30 mg/kg group. In fetuses, decreases in the numbers of implantations and live embryos and increases in the pre- and post-implantation losses were evident in the 30 mg/kg group. These changes were considered to be due to the inherent pharmacological action of PROJECT 7 (estrogen secretion inhibitory action via GnRH antagonist). All of the changes observed following administration of 600 mg/kg for 2 weeks prior to mating and during the mating period were recovered after 6-week recovery period, however, an abnormal estrus cycles were remained one of 20 rats. The NOAELs of PROJECT 7 were considered to be 600 mg/kg/day for general toxicity in female parental animals and 1 mg/kg/day for reproductive function of female parental animals and for early embryonic development.

* + - * 1. **Effects on Fertility in Female Rats (Treatment in the early stage of pregnancy)**

This study was designed to evaluate the potential adverse effects of PROJECT 7 on early embryonic development when administered orally to 19 or 20 pregnant rats/group at dose levels of 0 (vehicle control), 1, 30, 100 and 600 mg/kg/day on Days 0-7 of gestation. In dams, transiently decreased food consumption after initiation of dosing and suppressed body weight gains during the dosing period were noted in the 600 mg/kg group. All female parental animals survived the duration of the study with no clinical signs or gross lesions. No treatment-related effects were evident on fertility or maintenance of pregnancy in any female parental animal. Decreased fetal weights suggestive of delayed implantation were noted in the 100 and 600 mg/kg groups. In fetuses, decreased fetal and placental weights and premature fetuses were noted in the 600 mg/kg group. Decreased fetal weights were also noted in the 100 mg/kg group. No treatment-related effects were evident on embryo-fetal viability.

The NOAELs of PROJECT 7 were considered to be 100 mg/kg/day for general toxicity in female parental animals and 30 mg/kg/day for reproductive function of female parental animals and for embryo-fetal development.

# Table 5 - 15 Reproductive Study: Summary of Effects on Fertility

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain Dosing**  **details** | **Sex/ No./ dose**  **group** | **Doses (mg/kg)** | **Death** | **Major findings** | **Study No.** |
| Effects on fertility in male rats | Rat, SD,  Oral, gavage | 20M  20M  20M  20M  20M  (recovery 20M) | 0,  1,  30,  100 a),  600 b) | None | F0 male:  600 mg/kg: food consumption ↓  F1 embryo: No change | Project 7-TX-  0026 |
| Effects on estrus cycle in female rats (DRF) | Rat, SD,  Oral, gavage | 6F  6F  6F  6F | 0,  3,  100,  600 | None | 600 mg/kg: body weight gain, food consumption ↑  ≥100 mg/kg: abnormal estrus cycle (not recovered completely in the 2- week recovery period) | Project 7-TX-  0011 |
| Effects on ovulation in female rats  (DRF) | Rat, SD,  Oral, gavage | 4F  4F  4F  4F | 0,  3,  100,  600 | None | ≥100 mg/kg: ovulation was completely inhibited. | Project 7-TX-  0028 |
| Effects on fertility in female rats – Treatment in the pre- mating period | Rat, SD,  Oral, gavage | 20F  20F  20F  20F  (recovery 20F) | 0,  1 c),  30,  600 d), | None | F0 dam:  ≥30 mg/kg: prolongation of estrus cycle,  pre-coital period and fertility index  ↓  600 mg/kg: copulation index ↓, non-pregnant animals ↑  F1 embryo:  30 mg/kg: No. of implantation and live embryo ↓  pre- and post-implantation loss ↑ | Project 7-TX-  0012 |
| Effects on fertility in female rats – Treatment in the early stage of pregnancy | Rat, SD,  Oral, gavage | 20F  20F  20F  19F  20F | 0,  1,  30 c),  100 d),  600 | None | F0 dam:  600 mg/kg: body weight gain, food consumption ↓  F1 fetus:  ≥100 mg/kg: body weight ↓ 600 mg/kg: placental weight ↓ | Project 7-TX-  0013 |

↓: decrease, ↑: Increase, NOAELs are showed with underline. M: male, F: female

1. : NOAEL for paternal general toxicity.
2. : NOAEL for paternal reproductive function and for early embryonic development.
3. : NOAEL for maternal reproductive function and for early embryonic development.
4. : NOAEL for maternal general toxicity.
   * 1. **Genotoxicity**

The genotoxicity potential of PROJECT 7 was evaluated in an *in vitro* reverse mutation test, an *in vitro* chromosomal aberration test, an *in vivo* micronucleus test, an *in vivo/in vitro* unscheduled DNA synthesis test. The results are summarized in [Table 5 - 16](#_bookmark50) and toxicokinetic data are shown in [Table 5 - 17](#_bookmark51).

* + - 1. ***In vitro* reverse mutation**

Mutagenic potential of PROJECT 7 was assessed in a bacterial reverse mutation assay using *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2*uvr*A. The test was conducted by the pre-incubation method in the presence and absence of S9 mix. The main test was conducted at doses of 39.1 - 2500 g/plate for TA100, TA1535, and TA1537, and at doses of 78.1 - 5000 g/plate for WP2*uvr*A and TA98 in the presence or absence of S9 mix.

PROJECT 7 did not induce gene mutation in bacteria at any dose.

* + - 1. ***In vitro* chromosomal aberration**

An *in vitro* chromosomal aberration study of PROJECT 7 was conducted using CHL/IU cells. Concentrations of 15 - 270 g/mL in the 6-hour assay without metabolic activation system (S9 mix) and 210 - 270 g/mL in the 6-hour assay with S9 mix and 15 - 90 g/mL in the 24- hour assay without S9 mix were selected for microscopic observation.

In the 6-hour assay without S9 mix, a significant increases in chromosomal numerical aberrations was noted at 60 - 240 g/mL and a significant increase in chromosomal structural aberrations was noted at 270 g/mL. In the 6-hour assay with S9 mix, a significant increase in chromosomal numerical aberrations was noted at 240 g/mL. In the 24-hour assay without S9 mix, a significant increase in chromosomal numerical aberrations was noted at 30 to 90

g/mL.

PROJECT 7 was considered to have the ability to induce chromosomal aberration *in vitro*.

* + - 1. ***In vivo* micronucleus study**

A micronucleus test was conducted in mice to assess the *in vivo* clastogenicity of PROJECT 7. PROJECT 7 solid dispersion was given to ICR mice (5/sex/group) in a single oral dosing at 0, 300, 600, and 1200 mg/kg as PROJECT 7. Peripheral blood was collected at 48 and 72 hour after dosing in order to count micronucleated reticulocytes.

The mean micronucleated reticulocyte incidence in the test article-treatment groups were similar to those in the vehicle control group, indicating that PROJECT 7 has no clastogenic potential *in vivo*.

* + - 1. ***In vivo/in vitro* unscheduled DNA synthesis**

PROJECT 7 was examined for its ability to induce unscheduled DNA synthesis (USD) in rat hepatocytes in order to evaluate the effects to induce DNA damage *in vivo*. PROJECT 7 solid dispersion was given orally to male and female SD rats (4/sex/group) at doses of 300, 600, and 1200 mg/kg as PROJECT 7 and hepatocytes were isolated and prepared to calculate net grains (NG) and percentage of cells in repair. UDS induction was examined using autoradiography method.

The data obtained from animals treated with the test substance were comparable to those in the negative control groups. PROJECT 7 have no ability to induce DNA damage *in vivo*.

# Table 5 - 16 Summary of Genotoxicity Study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study** | **Species,**  **Strain, Dosing** | **Doses** | **Major findings** | **Study No.** |
| Reverse | *S. typhimurium* | TA100, TA1535, TA1537: | No increase in the number of revertant | Project 7- |
| mutation test | and *E. coli* | 39.1, 78.1, 156, 313, 625, | colonies | TX- |
|  |  | 1250, 2500 g/plate |  | 0001 |
|  |  | WP2uvr, TA98: |  |  |
|  |  | 78.1, 156, 313, 625, 1250, |  |  |
|  |  | 2500, 5000 g/plate |  |  |
| Chromosomal | CHL/IU cells | 6h (S9-): | The incidence of cells with chromosomal numerical aberrations was noted at 60 to 240 g/mL in the 6h (S9-) assay, at 240  g/mL in the 6h (S9+) assay, and at 30 to 90 g/mL in the 24h (S9-) assay.  The incidence of cells with chromosomal structural aberrations were noted at 270  g/mL in the 6h (S9-) assay. | Project 7- |
| aberration test |  | 15, 30, 60, 90, 120, 150, | TX- |
|  |  | 180, 210, 240, 270 g/mL | 0002 |
|  |  | 6h (S9+): |  |
|  |  | 210, 240, 270 g/mL |  |
|  |  | 24h (S9-): |  |
|  |  | 15, 30, 60, 90 g/mL |  |
| Micronucleus | Mouse, ICR | 0, 300, 600, 1200 mg/kg | No significant difference was noted in the | Project 7- |
| test |  |  | incidence of micronucleated reticulocytes | TX- |
|  |  |  | in the treated group when compared with | 0014 |
|  |  |  | that of control. |  |
| Unscheduled | Rat, SD | 0, 300, 600, 1200 mg/kg | No increase in percentage of cells in | Project 7- |
| DNA synthesis |  |  | repair | TX- |
| test |  |  |  | 0015 |

S9-: without S9 mix, S9+: with S9 mix

# Table 5 - 17 Summary of Toxicokinetics Data in a micronucleus study in mice dosed at 1200 mg/kg

|  |  |  |
| --- | --- | --- |
| **Sex** | **Cmax (ng/mL)** | **AUC0-24h (ng.h/mL)** |
| Male | 102000 | 975740 |
| Female | 139000 | 1050030 |

* 1. **Integrated Discussion of Nonclinical Data**

Suppression of the hypothalamic-pituitary-gonadal axis via GnRH receptor for the management of sex-steroid-dependent diseases (such as precocious puberty, prostate cancer, endometriosis and uterine fibroids) is a proven concept in clinical practice. PROJECT 7 is a potent non-peptide GnRH receptor antagonist and can be developed for the treatment of prostate cancer and endometriosis.

Although there is a high (>80%) homogeneity for GnRH receptors in various species, most non-peptide antagonists exhibit species selectivity which complicates the extrapolation of animal data to human. PROJECT 7 is no exception. Studies showed that PROJECT 7 had the highest affinity for the human GnRH receptor (IC50 = 0.1 nM) and the drug potency was 22-, 3.9-, and 36-fold lower with rat, monkey and dog GnRH receptors, respectively. In a functional assay measuring intracellular calcium concentrations ([Ca2+]i), PROJECT 7 exhibited potent antagonistic activity (IC50 = 2.9 nM) on GnRH-stimulated increases in [Ca2+]i, with similar species specificity observed.

Most of the pharmacology studies were performed in male animals, either intact or castrated. Despite the in vitro data indicating reduced affinities for GnRH receptors in these species compared to the human receptor, PROJECT 7 was able to show significant inhibitions on LH and testosterone levels after oral administration. In ovariectomized rats, similar inhibitory effects on LH levels were obtained, suggesting PROJECT 7 would be effective in both sexes.

It was observed that the effects of single doses of PROJECT 7 were reversible in nature since the magnitude of inhibition decreased with time within the 24-h period. Though not studied in pharmacological studies, the reversibility of GnRH antagonism after repeated daily dosing of PROJECT 7 was strongly indicated by the observations in animal toxicity studies. In female rats receiving 4-week daily dosing, the changes in sex organs were completely recovered after a 4-week untreated recovery period.

In monkey and dog pharmacology studies, a clear dose-dependent response could not be obtained. This may be attributed by factors such as short elimination t1/2 (a few hours in dogs), non-linear PK in monkeys, and susceptibility of T levels to stress and experimental procedures.

The pharmacokinetics in rats and dogs has been studied. Absolute bioavailabilities were 61.2%-36.7% (males) and 96.8%-50.2% (females) in rats and 23.9%-12.8% (males) and 36.4%-16.0% (females) in dogs. Cmax and AUC0-24h increased with the increment of the doses, however, less than dose proportional in rats and dogs. TK data on multiple oral dosing was collected during the 4 week toxicity studies in rats and dogs. Although Cmax and AUC0- 24h at all doses tested (1, 30, 100, and 600 mg/kg) in rats were not changed after repeated dosing, doses of 50 and 300 mg/kg in dogs markedly decreased both of these parameters following 2 week or more repeated dosing.

The *in vitro* metabolic fingerprinting studies using liver microsomes and cryopreserved hepatocytes showed no human specific metabolites supporting the selection of rats and dogs as the test species used in the toxicity studies.

Results of *in vivo* and *in vitro* metabolic fingerprinting studies demonstrated that PROJECT 7 is metabolized by mainly glucuronidation, and to a lesser extent by CYP-mediated oxidative metabolism.

Metabolism studies showed that CYP3A4/5 was the CYP isozyme mainly involved in the metabolism of PROJECT 7 in humans and that PROJECT 7 has a low inhibitory potential towards major CYP isozymes.

Morality was not encountered in the single oral dose toxicity studies in rats and dogs up to the highest doses. These doses were based on the most relevant concentration of PROJECT 7 in solid dispersion formulation to enhance its absorption and the maximum volume that can be given to the animals. The high doses were achieved by duplicate dosing in both rats and dogs. Approximately lethal dose of PROJECT 7 was estimated to be greater than 1200 mg/kg for rats and greater than 600 mg/kg for dogs.

No particular dose-limiting toxicity was observed in nonclinical safety studies. Loose, muddy or watery stools were observed in dogs at 50 mg/kg or more. Vomitus was also evident in dogs at 300 mg/kg, in most cases, during 1-6 hours after dosing at frequency of 1-6 days out of 28 days. These findings suggest that GI tract is a target organ of toxicity of PROJECT 7.

Decreases in RBC, HGB or HCT observed in this study at 300 mg/kg maybe due to

decreased testosterone levels, since testosterone enhances erythropoietin production, however, this consideration is unclear because no effects on reproductive organs were noted in this study. In the 4-week toxicity study in rats, increases in total cholesterol, phospholipids, Ca, total protein or albumin, were slight and without possible relative histopathological findings. Although increases in liver weights were noted both in rats and dogs, these findings were considered to be of minimal toxicological significance due to the absence of related histopathological lesions.

In the 4-week toxicity study, several findings related to pharmacological effects of PROJECT 7 were noted in female rats. An ovulation disorder observed in rats was suggested by the presence of ovarian follicular cysts and the decreased corpora lutea with decreased ovary weights.

On the other hand, changes that were significant enough to suggest an association with the pharmacological effects were not noted even at the highest dose in male rats or dogs.

Although decreased seminal vesicle weight was noted at the highest dose in the 4-week study in rats, no morphological changes suggesting the pharmacological effect were noted in male rats or dogs in the 4-week toxicity studies. In addition, there were no male toxicity on genital organs, sperm evaluation, mating, fertility or pre-coital period in days in the male fertility study in rats. Taking together with no obvious changes noted in male sex organs in the 4- week study in rats, it is considered that a more sustained and effective GnRH receptor blockade maybe necessary for the induction of clear changes to the male reproductive systems. From this aspect, the lack of sex organ-related changes except decreased weight of seminal vesicle might be linked with the observation that plasma concentrations of the C24h values, even at the highest doses (597-1110 ng/mL for rats; 8–30 ng/mL for dogs), were below the effective plasma concentration for lowering testosterone level to that of castration

(approximately 1400 ng/mL for rats; approximately 150 ng/mL for dogs) in the pharmacology studies.

In reproductive toxicity studies, several changes considered to be associated with the pharmacological effects of PROJECT 7 were seen in female rats, such as prolongation of estrus cycle, decreased number of corpora lutea with decreased ovary. A decrease in copulation index, shortening of pre-coital period, increased pre- and post-implantation loss, decreases in fertility indices were noted in female fertility studies. A decrease in number of live embryos, a decrease in fetal body, and delayed ossification were observed in female fertility studies or embryo fetal toxicity studies. These changes are considered to be due to imbalance of progesterone and estrogen based on GnRH inhibitory activity, decreased estrogen levels, or reflect the secondary effects of decreased hormone levels such as abnormal intrauterine environment or delayed implantation. No teratogenic findings were observed in the embryo- fetal development toxicity studies in rats or rabbits.

PROJECT 7 did not induce gene mutation in bacteria in an *in vitro* reverse mutation test, but induced chromosomal aberration in an *in vitro* chromosomal aberration test. However, PROJECT 7 did not affect on clastogenicity in an *in vivo* micronucleus test in mice at the high dose of 1200 mg/kg or on DNA damage in an *in vivo* unscheduled DNA synthesis test in rats at the high dose of 1200 mg/kg. Therefore, PROJECT 7 is considered not to exert a harmful influence on gene *in vivo*, or in humans.

In conclusion, the non-clinical data package is suitable and sufficient to support the initiation of FIM studies up to four weeks duration in male healthy volunteers, and those in female healthy volunteers with restriction that they are either not of childbearing potential or are practicing effective measures of contraception.

* 1. **References**

Not applicable