* 1. **Safety Pharmacology Studies**

PROJECT T had no effects on the central nervous system (physical condition and behavior) in male rats at doses up to 2000 mg/kg [Project T-PT-0001].

PROJECT T exerted no effects on the respiratory system (respiratory rate, tidal volume, and minute volume) in male rats at doses up to 2000 mg/kg [Project T-PT-0002].

PROJECT T exhibited no effects on the heart rate, blood pressure (systolic blood pressure, diastolic blood pressure, and mean blood pressure) or the electrocardiogram parameters (PR interval, QRS duration, QT interval, and QTc interval) in male dogs at up to 2000 mg/kg [Project T-PT-0003].

In HEK293 cells transfected with hERG channel, PROJECT T showed no effects on the hERG current at up to 1×10-4 mol/L [Project T-PT-0004].

## Table 4-4 Safety Pharmacology Studies of PROJECT T

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Study type | Test system | Animal species, strain, sex,  dosage regimen | Variable | Major  finding | Study No. |
| Central nervous system | FOB | Rats, SD, male  6 animals in each dose group 0, 20, 200, 2000 mg/kg | Physical condition and behavior | No change | Project T-PT-  0001 |
| Respiratory system | Whole body plethysmo-  graphy | Rats, SD, male  8 animals in each dose group 0, 20, 200, 2000 mg/kg | Respiratory rate, tidal volume, and minute volume | No change | Project T-PT-  0002 |
| Cardiovascular system | Telemetry method | Dogs, beagle, male  4 animals in each dose group 0, 20, 200, 2000 mg/kg | Heart rate, blood pressure, (systolic blood pressure, diastolic blood pressure, and mean blood pressure) and electrocardiogram (PR interval, QRS duration, QT  interval, and QTc interval) | No change | Project T-PT-  0003 |
| hERG current | Whole-cell patch clamp technique | HEK293 cells transfected with hERG channel  5 samples  0, 1×10-6, 1×10-5,  1×10-4 mol/L | Inhibitory effects on hERG current | No change | Project T-PT-  0004 |



1. **NONCLINICAL STUDIES: TOXICITY**
   1. **List of Studies**

A list of studies conducted is show[n in Table 6-1.](#_bookmark60)

## Table 6-1 List of Toxicity Studies

|  |  |  |  |
| --- | --- | --- | --- |
| Type of  study | Study title | GLP/  Non-GLP | Study No. |
| Single-dose | Single oral dose toxicity study in rats | GLP | Project T-TX-0001 |
| Single oral dose toxicity study in dogs | GLP | Project T-TX-0002 |
| Repeated- dose | 4-week repeated oral dose toxicity study in rats (with 4-week  recovery) | GLP | Project T-TX-0003 |
| 4-week repeated oral dose toxicity study in dogs (with 4-week  recovery) | GLP | Project T-TX-0004 |
| Additional high-dose, 4-week repeated oral dose toxicity study  in rats (with 4-week recovery) | GLP | Project T-TX-0014 |
| Additional high-dose, 4-week repeated oral dose toxicity study  in dogs (with 4-week recovery) | GLP | Project T-TX-0013 |
| Genotoxicity | Bacterial reverse mutation study | GLP | Project T-TX-0005 |
| Chromosome aberration test using cultured mammalian cells | GLP | Project T-TX-0006 |
| Rat micronucleus test | GLP | Project T-TX-0007 |
| Unscheduled DNA synthesis test in rat hepatocytes | GLP | Project T-TX-0012 |
| Reproduction toxicity | Study of fertility and early embryonic development to  implantation in rats | GLP | Project T-TX-0016 |
| Study for effects on embryo-fetal development in rats (dose-  range finding study) | Non-GLP | Project T-TX-0008 |
| Study for effects on embryo-fetal development in rats | GLP | Project T-TX-0009 |
| Study for effects on embryo-fetal development in rabbits (dose-  range finding study) | Non-GLP | Project T-TX-0010 |
| Study for effects on embryo-fetal development in rabbits | GLP | Project T-TX-0011 |
| Other studies | 4-week repeated oral dose combination toxicity study with  tamsulosin in rats | GLP | Project T-TX-0015 |

* 1. **Single-Dose Toxicity Studies**

Acute toxicity of PROJECT T was assessed using rats and dogs. PROJECT T was given as a single oral dose at 500 and 2000 mg/kg in rats and at 500, 1000, and 2000 mg/kg in dogs, followed by observation of the animals for a total of 15 days, including the day of administration, up to 14 days post-dose ([Table 6-2](#_bookmark62)).

Because no rats or dogs died at doses up to 2000 mg/kg, the highest dose, the lethal dose was estimated more than 2000 mg/kg.

In rats, there were no changes at doses up to 2000 mg/kg, the highest dose [Project T-TX-0001]. In dogs, no changes were observed at doses up to 2000 mg/kg apart from yellowish white feces considered to contain unabsorbed test article were found in animals in all treatment groups on the day of administration and the next day [Project T-TX-0002].

[Table 6-3](#_bookmark63) shows the results of plasma concentrations. The Cmax of both male and female rats increased as the dose increased, while AUC24h after administration of 500 mg/kg was not different from that of 2000 mg/kg in both male and female rats. The Cmax and AUC24h of female rats were greater than those of male rats. In dogs, the Cmax and AUC24h did not show dose dependency.

## Table 6-2 Single-Dose Toxicity Studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Type of study | Animal species, strain, details of  administration | No. of animals/sex/  group | Dose (mg/kg) | Death | Finding | Study No. |
| Single- dose | Rats, SD, oral  gavage, 15-day observation | 5 males and  5 females | 0  500  2000 | None | None | Project T-TX-  0001 |
| Single- dose | Dogs, beagle, oral gavage, 15- day observation | 1 male and  1 female | 500  1000  2000 | None | Yellowish white feces considered to contain the test  article | Project T-TX-  0002 |

## Table 6-3 Summary Table of Exposures in Single-Dose Toxicity Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Study title | Dose  (mg/kg) | Sex | Cmax (ng/mL) | AUC24h  (ng·h/mL) | Study No. |
| Single oral dose toxicity study in rats | 500 | Male  Female | 3130  5620 | 32234  40517 | Project T-TX-0001 |
| 2000 | Male  Female | 5550  10700 | 32006  39379 |
| Single oral dose toxicity study in dogs | 500 | Male  Female | 1700  910 | 6460  5988 | Project T-TX-0002 |
| 1000 | Male  Female | 899  1680 | 5095  12098 |
| 2000 | Male  Female | 1150  638 | 5542  3601 |

* 1. **Repeated Dose Toxicity**

Repeated-dose toxicity studies of PROJECT T were conducted in rats and dogs.

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

A 4-week repeated oral dose toxicity study of PROJECT T was performed in rats at doses of 100, 300 and 1000 mg/kg/day ([Table 6-4](#_bookmark65)).

No effects were observed in general condition, body weight, food consumption, water consumption, ophthalmology, urinalysis, hematology, blood biochemistry, necropsy, organ weights, or histopathology at doses up to 1000 mg/kg/day [Project T-TX-0003]. Therefore, the NOAEL (no observed adverse effect level) was concluded to be 1000 mg/kg/day.

The results of plasma concentrations are shown i[n Table 6-5.](#_bookmark66) The Cmax increased as the dose increased, though with saturation tendency, in both female and male animals. The AUC24h tended to saturate in both females and males with no dose-dependency. The AUC24h after the final dose tended to be greater than that after the first dose.

## Additional High-Dose, 4-Week Repeated Oral Dose Toxicity Study in Rats with 4-Week Recovery

To evaluate toxicity of PROJECT T at higher dose levels, a 4-week oral dose toxicity study of PROJECT T in rats was conducted at doses of 1000 and 2000 mg/kg/day (1000 mg/kg twice daily) ([Table 6-4](#_bookmark65)).

No effects were observed in body weight, food consumption, water consumption, ophthalmology, urinalysis, hematology, blood chemistry, necropsy, or organ weights at doses up to 2000 mg/kg/day. White feces were observed in male and female rats at a dose of 2000 mg/kg/day, but this finding was judged to have no toxicological significance, because it was considered to be the result of excretion of unabsorbed test article. On histopathology, the incidences of diffuse thickening and cell infiltration of the cecal mucosa increased in males at doses of 1000 and 2000 mg/kg/day. However, such changes were also observed in the control groups and were not degenerative changes, and they might be physiological reactions related to a slight increase in the activity of gut-associated lymphatic tissue (GALT) due to the presence of a large amount of the test article in the gastrointestinal tract. These findings were considered to be of little toxicological significance [Project T-TX-0014]. Therefore, the NOAEL was concluded to be 2000 mg/kg/day.

The results of plasma concentrations are shown i[n Table 6-5](#_bookmark66) and [Table 6-6.](#_bookmark67) The Cmax of PROJECT T and its metabolite, URM-1 (AS2780148-00) in the 1000 mg/kg/day group was almost comparable to that in the 2000 mg/kg/day group. The AUC24h of both PROJECT T and AS2780148-00 increased with increasing dose levels. There were no effects of repeated administration, and no gender differences were observed.

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

A 4-week repeated oral dose toxicity study of PROJECT T was performed in dogs at doses of 30, 100 and 300 mg/kg/day ([Table 6-4](#_bookmark65)).

Apart from yellowish white feces considered to contain unabsorbed test article were observed at 100 and 300 mg/kg/day, no effects were observed in body weight, food consumption, ophthalmology, urinalysis, hematology, blood biochemistry, necropsy, organ weights, or histopathology at doses up to 300 mg/kg/day [Project T-TX-0004]. Therefore, the NOAEL was concluded to be 300 mg/kg/day.

The results of plasma concentrations are shown i[n Table 6-5.](#_bookmark66) The Cmax and AUC24h showed dose-dependency at doses up to 100 mg/kg/day while saturation tendency was observed at, 300 mg/kg/day. There were no effects of repeated administration or sex differences observed.

## Additional High-Dose, 4-Week Repeated Oral Dose Toxicity Study in Dogs with 4-Week Recovery

To evaluate toxicity of PROJECT T at higher dose levels, a 4-week repeated oral dose toxicity study of PROJECT T in dogs was conducted at doses of 1000 and 2000 mg/kg/day (1000 mg/kg twice daily) ([Table 6-4](#_bookmark65)).

Soft stools were observed in males and females at a dose of 2000 mg/kg/day.

Feces containing a white test article-like substance were observed in males and females at doses of 1000 and 2000 mg/kg/day, but this finding was judged to have no toxicological significance, because it was probably due to excretion of unabsorbed test article into stools. In addition, no effects were observed in body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, organ weights, necropsy, or histopathology at doses up to 2000 mg/kg/day [Project T-TX-0013]. Therefore, the NOAEL was concluded to be 1000 mg/kg/day.

The results of plasma concentrations are shown i[n Table 6-5](#_bookmark66) and [Table 6-6.](#_bookmark67) The Cmax of PROJECT T and its metabolite, AS2780148-00, in the 1000 mg/kg/day group was almost comparable to that in the 2000 mg/kg/day group. The AUC24h of both PROJECT T and AS2780148-00 increased with increasing dose levels. There were no effects of repeated administration, and no gender differences were observed.

## Table 6-4 Repeated Dose Toxicity Studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Study title | Animal species, strain, details of  administration | No. of animals/sex/  group | Dose (mg/kg/day) | Death | Finding | Study No. |
| 4-week repeated oral dose study in rats (with 4- week recovery) | Rats, SD, oral gavage | 10 males and  10 females  7 males and  7 females (recovery) | 0  100  300  1000 | None1) | None | Project T-TX-  0003 |
| Additional high- dose, 4-week repeated oral dose study in rats (with 4- week recovery) | Rats, SD, oral gavage | 10 males and  10 females  5 males and  5 females (recovery) | 0  1000  2000  (1000×2) | None | White feces considered to contain the test article, increased incidences of diffuse thickening and cell infiltration  of the cecal mucosa | Project T-TX-  0014 |
| 4-week repeated oral dose study in dogs (with 4- week recovery) | Dogs, beagle, oral gavage | 3 males and  3 females  2 males and  2 females (recovery) | 0  30  100  300 | None | Yellowish white feces considered to contain the test  article | Project T-TX-  0004 |
| Additional high- dose, 4-week repeated oral dose study in  dogs (with 4- week recovery) | Dogs, beagle, oral gavage | 4 males and  4 females  3 males and  3 females (recovery) | 0  1000  2000  (1000×2) | None | Soft stools, feces containing white test  article-like substance | Project T-TX-  0013 |

1) Two control animals, 1 animal in the 100 mg/kg group, and 1 animal in the 300 mg/kg group died due to intubation error.

The underlined are the NOAELs.

## Table 6-5 Summary Table of Exposures in Repeated Dose Toxicity Studies

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Study title | Dose (mg/kg/  day) | Sex | Cmax (ng/mL) | | AUC24  (ng·h/mL) | | Study No. |
| First dose | Final dose | First dose | Final dose |
| 4-week repeated oral dose study in rats (with 4-week recovery) | 100 | Male  Female | 1680  2150 | 1420  1640 | 9303  10817 | 12542  13273 | Project T-TX-  0003 |
| 300 | Male  Female | 2890  2980 | 1690  3840 | 12185  11420 | 18058  15946 |
| 1000 | Male  Female | 3580  4010 | 2230  4490 | 11867  12162 | 16777  24971 |
| Additional high- dose, 4-week repeated oral dose study in rats (with  4-week recovery) | 1000 | Male  Female | 4367  5433 | 4528  6618 | 11963  10084 | 17479  16581 | Project T-TX-  0014 |
| 2000  (1000×2) | Male  Female | 5161  5579 | 4474  7196 | 27696  24647 | 34455  31996 |
| 4-week repeated oral dose study in dogs (with 4-week recovery) | 30 | Male  Female | 485  473 | 366  625 | 3113  3018 | 3217  3014 | Project T-TX-  0004 |
| 100 | Male  Female | 1060  874 | 914  1020 | 6250  6941 | 8009  6938 |
| 300 | Male  Female | 1350  886 | 1190  1140 | 9182  5919 | 8044  6970 |
| Additional high- dose, 4-week repeated oral dose study in dogs  (with 4-week recovery) | 1000 | Male  Female | 769  1183 | 850  1217 | 4581  5764 | 5156  4878 | Project T-TX-  0013 |
| 2000  (1000×2) | Male  Female | 1288  1089 | 1244  1240 | 8122  9658 | 9101  8901 |

The underlined are the NOAELs.

## Table 6-6 Summary Table of Exposures in Repeated Dose Toxicity Studies (AS2780148-00)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Study title | Dose (mg/kg/  day) | Sex | Cmax (ng/mL) | | AUC24  (ng·h/mL) | | Study No. |
| First dose | Final dose | First dose | Final dose |
| Additional high- dose, 4-week repeated oral dose study in rats (with  4-week recovery) | 1000 | Male  Female | 13922  13114 | 11684  11694 | 82325  71752 | 94428  95286 | Project T-TX-  0014 |
| 2000  (1000×2) | Male  Female | 17017  12793 | 12996  12956 | 165074  145380 | 147226  157573 |
| Additional high- dose, 4-week repeated oral dose study in dogs  (with 4-week recovery) | 1000 | Male  Female | 1540  1751 | 1515  1667 | 9924  11649 | 10939  9963 | Project T-TX-  0013 |
| 2000  (1000×2) | Male  Female | 1831  1699 | 1748  1596 | 22673  22200 | 23117  19861 |

The underlined are the NOAELs.

* 1. **Genotoxicity Studies**

In a reverse mutation study using *Salmonella typhimurium* (TA100, TA1535, TA98, TA1537) and *Escherichia coli* (WP2*uvrA*), the reverse mutation colonies did not increase for TA100, TA98, and WP2*uvrA* at concentrations up to 5000 µg/plate and for TA1535 and TA1537 at concentrations up to 2500 µg/plate with or without metabolic activation [Project T- TX-0005].

In a chromosome aberration test using pneumocytes of Chinese Hamster (CHL/IU cells), the incidence of structural chromosome aberrations increased after short-time (6 hours) at

500 µg/mL without metabolic activation, long-time (24 hours) at 250 µg/mL without metabolic activation, and short-time (6 hours) at 375 and 500 µg/mL with metabolic activation [Project T-TX-0006]. Precipitation of the test article was observed at all these doses at which the incidence of aberration increased.

In a rat micronucleus test (oral administration of 500, 1000, or 2000 mg/kg/day in rats for 2 days) [Project T-TX-0007], micronucleus induction was not observed even at doses at which growth of bone marrow cells was inhibited.

In an unscheduled DNA synthesis (UDS) test in rat hepatocytes (single oral administration of 500, 1000 or 2000 mg/kg/day in rats), there was no increase in the net nuclear grain count or the incidence of repair cells in isolated hepatocytes [Project T-TX-0012].

Although PROJECT T was showed the positive result in an *in vitro* chromosome aberration test, the result of the reverse mutation study was negative and no genotoxicity was observed in the rat micronucleus test and an unscheduled DNA synthesis (UDS) test using rat hepatocytes.

Thus, PROJECT T was judged to not exhibit genotoxicity under *in vivo* conditions.

## Table 6-7 Genotoxicity Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Type of study | Animal species, strain, details of administration | No. of animals/  sex/group | Dose | Major finding | Study No. |
| Reverse mutation study | *Salmonella typhimurium* (TA100, TA1535, TA98, TA1537)  *Escherichia coli*  (WP2*uvrA*) | - | 39.1-5000 µg/plate | No increase of reverse mutation colonies | Project T-TX-  0005 |
| Chromosome aberration test | CHL/IU cells Treatment for 6 hours with or without metabolic activation (S9), and treatment for 24 hours without S9. | - | 6 hours (without S9): 125-500 µg/mL  6 hours (with S9): 62.5-500 µg/mL  24 hours  (without S9): 62.5-250 µg/mL | Incidence of structural chromosome aberrations increased after 6-hour at 500 µg/mL without S9, 24-hour at 250 µg/mL without S9, and 6-hour at  375 and 500 µg/mL with S9. | Project T-TX-  0006 |
| Micronucleus test | Rat, SD, 2-day oral gavage | 6 males | 0  500  1000  2000 (mg/kg/day) | No increase of polychromatic erythrocytes with  micronucleus in femoral bone marrow cells. | Project T-TX-  0007 |
| Unscheduled DNA  synthesis test | Rat, SD, single oral gavage | 3 males | 0  500  1000  2000 (mg/kg/day) | No increase in the net nuclear grain count in the isolated hepatocytes or the incidence of repair cells  among them | Project T-TX-  0012 |

* 1. **Carcinogenicity Studies**

Not conducted.

* 1. **Reproduction Toxicity Studies**

## Study of Fertility and Early Embryonic Development to Implantation

Effects of PROJECT T on fertility and early embryonic development to implantation were evaluated in male and female rats at doses of 100, 1000 and 2000 mg/kg/day (1000 mg/kg twice daily) ([Table 6-8](#_bookmark71)).

In the study of the effects of PROJECT T on fertility and early embryonic development to implantation, not only were no effects observed on the frequency of estrus, estrous interval, the copulation rate, copulatory interval, fertility rate, and the number of corpora lutea, but also on the number of implantations, preimplantation deaths, postimplantation deaths, and live embryos. In dams at doses of 1000 and 2000 mg/kg/day, white feces potentially attributable to the presence of unabsorbed test article were observed [Project T-TX-0016].

Based on the above, the NOAEL of PROJECT T on fertility and early embryonic development in males and females was concluded to be 2000 mg/kg/day.

## Study for Effects on Embryo-Fetal Development

Studies for effects on embryo-fetal development were conducted in rats and rabbits. The results showed that PROJECT T has no teratogenicity in rats at doses up to 2000 mg/kg/day (1000 mg/kg twice daily) and in rabbits at doses up to 30 mg/kg/day ([Table 6-8](#_bookmark71)).

In rats, no effects were observed in a dose-range finding study at doses up to 2000 mg/kg/day [Project T-TX-0008]; in the pivotal study where the test article was given at 100, 1000, and

2000 mg/kg/day, no effects were observed in dams or embryo-fetal development at doses up to 2000 mg/kg/day, except for white feces in dams at doses of 1000 and 2000 mg/kg/day probably due to the excretion of unabsorbed test article into stools; and no teratogenicity was observed [Project T-TX-0009]. Based on the above, the NOAEL of PROJECT T was concluded to be 2000 mg/kg/day for both dams and embryo-fetal development.

For rabbits, in the dose-range finding study where the test article was given to non-pregnant rabbits at doses of 3, 30 and 300 mg/kg/day, decreases in body weight and food consumption were observed at a dose of 300 mg/kg/day; increases in AST, ALT, total bilirubin, total cholesterol, triglycerides, urea nitrogen, creatinine, and inorganic phosphorus and decreases in calcium, potassium, and chloride were observed in blood chemistry on Day 5; and discoloration of the kidneys and greenish liver were noted in necropsy. In histopathology of the kidney and liver, hypertrophy and vacuolation of hepatocytes, hypertrophy of bile duct epithelial cells, necrosis of proximal renal tubule epithelial cells, dilatation of the distal renal tubule, regeneration of the renal tubule, hyaline casts, and mononuclear cell infiltration were observed in this group. At a dose of 30 mg/kg/day, no test article related changes were noted [Project T-TX-0010]. In the dose-range finding study in pregnant rabbits, decreases in stool volume, no-stool and/or emaciation were observed at a dose of 100 mg/kg/day, and 2 of 6 rabbits died, and the remaining 4 were sacrificed due to the moribund condition for necropsy. Necropsy of animals sacrificed due to the moribund condition showed discoloration of the kidneys. At the same dose, decreases in body weight and food consumption were also observed. At a dose of 30 mg/kg/day, no test article related changes were observed [Project T- TX-0010]. Therefore, for the pivotal study in rabbits, the doses were set at 3, 10, and

30 mg/kg/day. In this rabbit study, abortion and decreases in body weight and food consumption were observed in dams at a dose of 30 mg/kg/day, while neither no effects on embryo-fetal development nor no teratogenicity was observed at doses up to 30 mg/kg/day [Project T-TX-0011]. Based on the above, the NOAEL of PROJECT T in rabbits was concluded to be 10 mg/kg/day for dams and 30 mg/kg/day for embryo-fetal development.

## Table 6-8 Reproduction Toxicity Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Study title | Animal species, strain, details of  administration | No. of animals/  sex/group | Dose (mg/kg/day) | Major finding | Study No. |
| Study of fertility and early embryonic development to implantation | Male rats, SD, oral gavage, from 4 weeks before mating through the mating  period until one day before necropsy | 20 males | 0  100  1000  2000  (1000×2) | White feces | Project T-TX-  0016 |
| Female rats, SD, oral gavage, from 2 weeks before mating through the mating period until day 7 of  gestation | 20 females | 0  100  1000  2000  (1000×2) | White feces | Project T-TX-  0016 |
| Study for effects on embryo-fetal development | Rats, SD, oral gavage, from day 7 to day 17 of gestation | 5-6 males | 0  100  1000  2000  (1000×2) | Dams White feces Fetus  No findings | Project T-TX-  0008 |
| 18-20  females | 0  100  1000  2000  (1000×2) | Dams White feces Fetus  No findings | Project T-TX-  0009 |
| Rabbits, New Zealand White, oral gavage, for 5 days | 3 non- pregnant females | 3  30  300 | Decreases in body weight and food consumption  Increases in AST, ALT, total bilirubin, total cholesterol, triglycerides, urea nitrogen, creatinine, and inorganic phosphorus, decreases in calcium, potassium, and chloride  Liver: Greenish, hypertrophy and vacuolation of hepatocytes, and hypertrophy of bile duct epithelial cells  Kidney: Discoloration, necrosis of proximal renal tubule epithelial cells, dilatation of the distal renal tubule, regeneration of the renal tubule, hyaline  casts, and mononuclear cell infiltration | Project T-TX-  0010 |
| Rabbits, New Zealand White, oral gavage, from day 6 to day 18 of gestation | 6 pregnant females | 0  10  30  100 | Dams  Death, decreases in body weight and food consumption  Fetus  No findings1) | Project T-TX-  0010 |
| 18-19  pregnant females | 0  3  102)  303) | Dams  Abortion, decreases in body weight and food consumption Fetus  No findings | Project T-TX-  0011 |

The underlined are the NOAELs.

1): Not evaluated at a dose of 100 mg/kg/day 2): NOAEL in dams 3): NOAEL in embryo-fetal development.

* 1. **Other Studies**

## 4-Week Repeated Oral Dose Combination Toxicity Study with Tamsulosin (YM617) in Rats

In the combination toxicity study of YM617 and PROJECT T, PROJECT T was repeatedly administered to rats at doses of 1000 and 2000 mg/kg/day (1000 mg/kg twice daily) in combination with YM617 at a dose of 30 mg/kg/day for 4 weeks.

In the YM617 alone group, incomplete eyelid opening, dilatation of the lumina in the seminal vesicles with increased weight, high columnar change of the endometrial epithelium in the uterus, and abnormal mucification of the surface epithelium in the vagina were observed. In the PROJECT T alone group, no toxicological findings were noted. In the PROJECT T and YM617 combination group, changes similar to those in the YM617 alone group were noted, but neither no enhancement of toxic changes nor renewed toxicity due to combined treatment were observed; and in exposure levels of both compounds, no obvious changes due to combined treatment were noted, either [Project T-TX-0015].

**6.8** **Discussions**

In nonclinical studies in rats, no toxicological findings related to PROJECT T were noted at doses up to 2000 mg/kg/day, which was set as the highest dose. In dogs, soft stools were observed at the highest dose of 2000 mg/kg/day, but neither a decrease in body weight nor changes in the gastrointestinal tract were observed. On the other hand, no toxicological findings were observed at a dose of 1000 mg/kg/day. Therefore, the NOAEL was concluded to be 2000 mg/kg/day for rats and 1000 mg/kg/day for dogs. The exposure at these NOAELs are show[n in Table 6-9.](#_bookmark74)

On the other hand, in the dose-range finding study in non-pregnant rabbits for effects on embryo-fetal development, increases in AST, ALT, total bilirubin, total cholesterol, triglycerides, urea nitrogen, creatinine, and inorganic phosphorus, and decreases in calcium, potassium, and chloride were observed at a dose of 300 mg/kg/day. Histopathology of the liver and kidneys showed hypertrophy and vacuolation of hepatocytes, hypertrophy of bile duct epithelial cells, necrosis of proximal renal tubule epithelial cells, dilatation of the distal renal tubule, regeneration of the renal tubule, hyaline casts, and mononuclear cell infiltration. Based on the above, it is expected that the liver and kidney might be the toxic target organ of PROJECT T, although no organ toxicity was observed in rats or dogs at the highest dose. The exposure at a dose of 300 mg/kg/day, at which these changes observed in non-pregnant rabbits, was much higher than that at the highest dose in rats and dogs ([Table 6-10](#_bookmark75)). Since the exposures in non-pregnant rabbits at 300 mg/kg/day was higher than that in humans at a dose of 400 mg, which was the highest dose for multiple administration in humans ([Table 6-11](#_bookmark76)), such changes in the kidney and liver are considered to not occur in multiple-dose studies in humans even at a dose of 400 mg.

In terms of the genotoxicity, although PROJECT T induced chromosome aberrations in the *in vitro* chromosome aberration test, the result of the reverse mutation study was negative, and

no genotoxicity was observed in the rat micronucleus test and the unscheduled DNA synthesis test using rat hepatocytes; thus, PROJECT T was judged to not exhibit genotoxicity under *in vivo* conditions.

In the reproductive toxicity, no effects on fertility and early embryonic development were observed in the study of fertility and early embryonic development in rats at doses up to 2000 mg/kg/day (1000 mg/kg twice daily), which was the highest dose. In studies for effects on embryo-fetal development in rats and rabbits, no effects on embryo-fetal development and no teratogenicity were observed at doses up to 2000 mg/kg/day (1000 mg/kg twice daily) and 30 mg/kg/day, respectively, which are both the highest doses.

In the 4-week repeated oral dose combination toxicity study with tamsulosin, neither no enhancement of toxic changes nor renewed due to combined treatment were observed.

## Table 6-9 Summary Table of Exposures at NOAELs in Nonclinical Toxicity Studies

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Study title | Dose (mg/kg/  day) | Sex | Cmax (ng/mL) | | AUC24h  (ng·h/mL) | | Study No. |
| First dose | Final dose | First dose | Final dose |
| Additional high- dose, 4-week repeated oral dose study in rats (with 4-  week recovery) | 2000 | Male | 5161  (45.93) | 4474  (39.82) | 27696  (246.49) | 34455  (306.65) | Project T-TX-  0014 |
| Female | 5579  (49.65) | 7196  (64.04) | 24647  (219.36) | 31996  (284.76) |
| Additional high- dose, 4-week repeated oral dose study in dogs (with 4-  week recovery) | 1000 | Male | 769  (7.23) | 850  (7.99) | 4581  (43.06) | 5156  (48.47) | Project T-TX-  0013 |
| Female | 1183  (11.12) | 1217  (11.44) | 5764  (54.18) | 4878  (45.85) |

The values in parentheses are the concentrations in the fraction unbound to plasma protein when the protein binding rate in rats is 99.11% and the protein binding rate in dogs is 99.06%.

## Table 6-10 Exposure in Non-pregnant Rabbits in a Dose-Range Finding Study (PROJECT T)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Study title | Dose (mg/kg/  day) | Sex | Cmax (ng/mL) | | AUC24h  (ng·h/mL) | | Study No. |
| First dose | Final dose | First dose | Final dose |
| Study for effects on embryo-fetal development in rabbits (non- pregnant rabbits  in a dose-range finding study) | 300 | Female | 66865  (193.91) | 192299  (557.67) | 1205344  (3495.50) | 4096164  (11878.88) | Project T-TX-  0010 |

The values in parentheses are the concentrations in the fraction unbound to plasma protein when the protein binding rate in rabbits is 99.71%.

## Table 6-11 Exposure after Multiple Administration in Humans (PROJECT T)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study title | Dose (mg) | Cmax (ng/mL) | AUC24h  (ng·h/mL) | Study No. |
| Multiple administration study in humans  (elderly, Day 10) | 400 | 1984  (5.75) | 14418  (41.81) | Project T-CL-0002 |

The values in parentheses are the concentrations in the fraction unbound to plasma protein when the protein binding rate in humans is 99.71%