### Safety Pharmacology

Studies investigating safety pharmacology of PROJECT W are listed in [[Table 2](#_bookmark29)]. PROJECT W has no effects on human ether-á-go-go-related gene (hERG) current or action potential duration up to 3 x 10-5 mol/L (9760 ng/mL, protein free condition).

In fasted rats, using the modified Irwin’s method, prone position, decreased locomotor activity and low level of reactivity were observed at 10 and 30 mg/kg. These findings indicated that PROJECT W has sedative effects. Low body temperatures were also recorded at the same doses.

In monkeys, palpebral ptosis, decreased BP and decreased body temperature were recorded at 10 and 30 mg/kg. HR and electrocardiography were not affected. With regard to respiratory functions, increased arterial carbon dioxide tension was recorded at 10 and 30 mg/kg. There were no alterations for the other respiratory functions such as respiration rate, arterial oxygen tension, hemoglobin oxygen saturation or arterial pH.

### Table 2 Safety Pharmacology Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Test System** | **Species, Strain, Sex, Dosing Particulars** | **End Point(s) Measured** | **Major Findings** | **Study No.** |
| **hERG current** | Whole cell | hERG channel | Inhibition of | No effects | Project W-PT-0001 |
|  | patch | transfected | hERG current |  |  |
|  | clamp | HEK293 cells |  |  |  |
|  |  | n = 5 |  |  |  |
|  |  | Concentrations: 0, |  |  |  |
|  |  | 3×10-7, 3×10-6, |  |  |  |
|  |  | 3×10-5 mol/L |  |  |  |
| **Action** | Glass | Papillary muscles | Resting | No effects | Project W-PT-0002 |
| **potential** | electrode | isolated from | membrane |  |  |
| **duration** | technique | Hartley guinea | potential, action |  |  |
|  |  | pigs | potential |  |  |
|  |  | n = 5 | amplitude, |  |  |
|  |  | Concentrations: 0,  3×10-7, 3×10-6,  3×10-5 mol/L | dV/dtmax, action potential duration  (APD30, APD90, |  |  |
|  |  |  | APD30–90) |  |  |
| **Cardiovascular** | Telemetry | Monkey, | General activity, |  10 mg/kg: Palpebral ptosis, decrease in blood pressure, increase in arterial carbon dioxide tension, decreased body temperature | Project W-PT-0003 |
| **and** |  | cynomolgus | behavior, body |  |
| **respiratory** |  | 4M | temperature, BP, |  |
| **system** |  | Doses: 0, 1, 3, 10, | HR, ECG |  |
|  |  | 30 mg/kg | parameters, |  |
|  |  |  | respiration rates, |  |
|  |  |  | blood-gas |  |
|  |  |  | parameters, |  |
|  |  |  | blood-electrolyte |  |
|  |  |  | concentrations |  |
| **Central nervous system** | Modified Irwin’s method | Rat, SD 6M  Doses: 0, 3, 10,  30 mg/kg | General activity, behavior, body temperature |  10 mg/kg: Prone position, decreased locomotor activity,  low level of | Project W-PT-0004 |
|  |  |  |  | reactivity, decrease in |  |
|  |  |  |  | body temperature |  |
|  |  |  |  | 30 mg/kg: Loss of |  |
|  |  |  |  | locomotor activity, |  |
|  |  |  |  | reddening skin |  |

APD: action potential duration; BP: blood pressure; dV/dtmax: maximum rate of rise of action potential; ECG: electrocardiogram; HEK293: human embryonic kidney cell line; hERG: human ether-á-go-go-related gene; HR: heart rate; SD: Sprague Dawley.

## Toxicology

### Single-dose Toxicity

Single-dose oral toxicity studies of PROJECT W were conducted at doses up to 300 mg/kg in rats and monkeys and are enumerated in [[Table 3](#_bookmark49)]. A dose of 300 mg/kg was intolerable in rats. Monkeys tolerated up to 300 mg/kg.

All rats at the highest dose of 300 mg/kg were sacrificed in extremis on the day of dosing (day 1) due to prone/lateral position, bradypnea and hypothermia. The lethal dose in rats, therefore, was judged as 300 mg/kg. Decreased spontaneous activity, transient body weight reduction, reddening skin and low body temperature were observed at lower doses. Corneal opacity was observed in a few females at 100 mg/kg. Microscopic examination showed epithelial desquamation and mineralization in the cornea. This was concluded to be caused by less frequent eye closure due to suppressed spontaneous activity.

There were no deaths in the monkey study up to 300 mg/kg. Decreased spontaneous activity, palpebral ptosis, eyelid closure, crouching, decreased food intake, mild alterations in hematology and blood chemistry, low body temperature and hypothermia-related electrocardiogram (ECG) changes were observed transiently.

### Table 3 Single-dose Toxicity Studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain,**  **Dosing Particulars** | **Sex/ No./ Dose** | **Doses (mg/kg)** | **Death** | **Major Findings** | **Study No.** |
| **Single- dose** | Rat, SD,  Oral gavage, observation for 14 days | 5M,  5F | 30 (F)  100  300 | 300 mg/kg (moribund sacrifice) on day 1 | Decreased spontaneous activity, transient body weight reduction, reddening skin, low body temperature, corneal opacity (100 mg/kg females) | Project W-TX-0001 |
| **Single- dose** | Monkey, cynomolgus, Oral gavage, observation for 14 days | 1M,  1F | 30  100  300 | No death | Decreased spontaneous activity, palpebral ptosis, eyelid closure, crouching, decreased food intake, mild alterations in hematology and blood chemistry, low body  temperature, hypothermia-related ECG changes | Project W-TX-0002 |

ECG: electrocardiogram; SD: Sprague Dawley.

### Repeat-dose Toxicity

Repeat-dose toxicity studies in rats and cynomolgus monkeys given PROJECT W are described in [[Table 4](#_bookmark53)].

### Rats

In Study Project W-TX-0003, rats were given PROJECT W orally for 4 weeks at the dose levels of 0, 1, 3, 10 and 30 mg/kg per day. These dose levels were determined from the results of a

1-week dose range finding study where the 30 mg/kg per day group showed a reduction in body weight [[Table 4](#_bookmark53)].

Spermatid retention in the seminiferous tubules at seminiferous cycle stages IX to XI was detected microscopically in all treatment groups. This suggests a disturbance of the final stage of spermatogenesis.

At 10 and 30 mg/kg per day, dose-dependent decreases in serum testosterone levels were observed. Histopathology examinations showed cellular hypertrophy in the adrenal cortical tissues (zona fasciculata and reticularis) and hypertrophy of the ovarian interstitial glands. These hypertrophic changes were slight and reversible and were considered to be associated with the alteration of glucocorticoids and sex hormones.

At 30 mg/kg per day, reddening auricle, weight reduction of the prostate and seminal vesicle, suppressed food intake and suppressed body weight gain were observed.

In addition, mild alterations in hematology, blood chemistry and urinalysis were recorded at 10 and 30 mg/kg per day.

All findings showed reversibility within 4 weeks after cessation of a 4-week administration.

Since this 4-week rat study failed to establish a NOAEL for males, a 4-week lower

dose-range study was conducted. Male rats were given PROJECT W orally at the dose levels of 0, 0.1 and 0.3 mg/kg per day. Spermatid retention was not observed in these dose levels.

NOAELs were judged as 0.3 and 3 mg/kg per day in male and female rats, respectively.

Rats were given PROJECT W orally for 13 weeks at the dose levels of 0, 0.1, 0.3, 1, 3 and 30 mg/kg per day (0.1 and 0.3 mg/kg were only given to males) (Study Project W-TX-0021). These dose levels were determined by the results of a 4-week study. A dose of 30 mg/kg, considered to be the maximum tolerable dose for 13-week repeated dosing, was set as the

high dose. Dose levels of 0.1 and 0.3 mg/kg for males and 1 mg/kg for females, expected to be NOAELs, were set as the low doses.

At 0.3 mg/kg and greater, spermatid retention in the seminiferous tubules at stages IX to XI of the seminiferous cycle in the testes and a high incidence of abnormal sperm (mainly no tail) were noted in males.

At 1 mg/kg and greater, low sperm count and low caudal epididymis weight were noted in males.

At 3 mg/kg and greater, low sperm activity rate, low epididymis weight and increased lipids in the zona fasciculata cells of the adrenal were noted in males.

At 30 mg/kg, reddening of the auricle, low body weight and food consumption, decreased body temperature, high adrenal weight and an increase in incidence of degenerative changes with brown secretion and/or mononuclear cell infiltration in the Harderian gland were noted in both sexes. Low eosinophil count and ratio, shortened prothrombin time and activated partial thromboplastin time, low motile sperm rate, small size of the epididymides, low seminal vesicle and prostate weights and high testis weight were noted in males. Low leukocyte count, enlargement of the adrenals, high ovary weight, increased lipids in the zona fasciculata cells of the adrenal and hypertrophy of the interstitial gland cells in the ovary were noted in females.

All findings, except for the Harderian gland changes showed reversibility within 4 weeks after cessation of a 13-week administration.

NOAELs were judged as 0.1 and 3 mg/kg per day in male and female rats, respectively.

### Monkeys

Monkeys were given PROJECT W orally for 4 weeks at doses of 0, 0.3, 1, 5 and 30 mg/kg per day (Study Project W-TX-0004). Cmax and AUC data from this study are shown in [[Table 8](#_bookmark68)].

These doses were determined from the results of a 1-week dose-range finding study where the 30 mg/kg per day group showed hypoactivity and suppressed food intake.

Treatment-related findings observed at the highest dose of 30 mg/kg per day were palpebral ptosis, decreased spontaneous activity, decreased food consumption and lower body temperature. Unlike rats, there were no alterations in the reproductive organs. The NOAEL in monkeys was judged as 5 mg/kg per day.

Monkeys were given PROJECT W orally for 13 weeks at the dose levels of 0, 1, 5 and 30 mg/kg per day (Study Project W-TX-0022). These dose levels were determined by the results of a

4-week study. A dose of 30 mg/kg, considered to be the maximum tolerable dose for

13-week repeated dosing, was set as the high dose. A dose level of 1 mg/kg, expected to be the NOAEL, was set as the low dose.

Treatment-related findings observed at the highest dose of 30 mg/kg per day were palpebral ptosis, decreased spontaneous activity, decreased food consumption and lower body temperature. Decreased serum testosterone levels were noted. Unlike rats, there were no alterations in the reproductive organs and semen analysis (sperm count, motile, and morphology). The NOAEL in monkeys was judged as 5 mg/kg per day.

### Table 4 Repeat-dose Toxicity Studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **Particulars** | **Sex/ No./ Dose** | **Doses (mg/kg)** | **Deaths** | **Major Findings** | **Study No.** |
| 1-week repeat- dose | Rat, SD,  Oral gavage | 4M, 4F | 0  1  **3** (M)  **10** (F)  30 | No death | 10 mg/kg: spermatid retention  30 mg/kg: decreased body weight, decreases in prostate and seminal vesicle weights, hypertrophy of adrenal cortex | Project W-TX-0024 Non-GLP) |
| 4-week repeat- dose with 4-week recovery | Rat, SD,  Oral gavage | 10M,  10F  5M, 5F  for recovery | 0  1  **3** (F)  10  30 | No death | Reproductive function related  1 mg/kg: spermatid retention  10 mg/kg: ↓ testosterone, hypertrophy of adrenal cortex (zona fasciculata and reticularis), hypertrophy of ovarian interstitial glands  30 mg/kg: ↓ prostate and seminal vesicle, ↑ adrenal weight | Project W-TX-0003 |
|  |  |  |  |  | Other changes  30 mg/kg: reddening auricles,  ↓ body weight gain, food consumption |  |
|  |  |  |  |  | Findings after recovery period 30 mg/kg: hypertrophy of ovarian interstitial glands  (only one female) |  |
| Additional  4-week repeat- | Rat, SD,  Oral gavage | 10M | 0  0.1  **0.3** (M) | No death | No findings | Project W-TX-0019 |
| dose |  |  |  |  |  |  |
| *Table continued on next page* | | | | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **Particulars** | **Sex/ No./ Dose** | **Doses (mg/kg)** | **Deaths** | **Major Findings** | **Study No.** |
| 13-week repeat- dose with 4-week recovery | Rat, SD,  Oral gavage | 10M  10F  5M, 5F  for recovery | 0  **0.1 (M)**  0.3  1  **3 (F)**  30 | No death | Reproductive function related  ≥ 0.3 mg/kg: spermatid retention, high incidence of abnormal sperm (mainly no tail)  ≥ 1 mg/kg: low sperm count, low caudal epididymis weight  ≥ 3 mg/kg: low sperm activity rate, low epididymis weight, hypertrophy of adrenal cortex (zona fasciculata and reticularis)  30 mg/kg:  prostate and seminal vesicle,  adrenal weight, low motile sperm rate, hypertrophy of ovarian intestinal glands | Project W-TX-0021 |
|  |  |  |  |  | Other changes  30 mg/kg: reddening auricles,   body weight gain, food consumption, degenerative changes with brown secretion and/or mononuclear cell infiltration in the Harderian gland |  |
|  |  |  |  |  | Findings after recovery period 30 mg/kg: hypertrophy of ovarian interstitial glands, low sperm activity, motile sperm rate and sperm count, high incidence of abnormal sperm (showed a tendency toward recovery), degenerative  changes with brown secretion in the Harderian gland |  |
| 1-week | Monkey, | 1M, 1F | 0 | No | 30 mg/kg: decreased | Project W-TX-0025 |
| repeat- dose | cynomolgus, Oral gavage |  | 1  **5**  30 | death | locomotor activity, ptosis, decreased food consumption, decreased rectal temperature | Non-GLP |
| *Table continued on next page* | | | | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **Particulars** | **Sex/ No./ Dose** | **Doses (mg/kg)** | **Deaths** | **Major Findings** | **Study No.** |
| 4-week repeat- dose with 4-week recovery | Monkey, cynomolgus, Oral gavage | 3M, 3F  3M, 3F  for recovery | 0  0.3  1  **5**  30 | No death | Reproductive function related Not observed  Other changes  30 mg/kg: decreased spontaneous activity, palpebral ptosis, low body temperature, ↓ food consumption | Project W-TX-0004 |
|  |  |  |  |  | Findings after recovery period No (All findings recovered) |  |
| 13-week repeat- dose with 4-week recovery | Monkey, cynomolgus, Oral gavage | 4M, 4F  3M, 3F  for recovery | 0  1  **5**  30 | No death | Reproductive function related 30 mg/kg:  testosterone  Other changes  30 mg/kg: decreased spontaneous activity, palpebral ptosis, low body temperature,  food consumption | Project W-TX-0022 |
|  |  |  |  |  | Findings after recovery period None (recovery occurred for all findings) |  |

Bold and underlined: NOAEL; GLP: Good Laboratory Practice; NOAEL: no observed adverse effect level; SD: Sprague Dawley; ↓: decreased; ↑: increased.

### Genotoxicity

Studies assessing genotoxic potential of PROJECT W are listed in [[Table 5](#_bookmark55)].

No increase in revertant colonies was noted on plates treated with PROJECT W in a bacterial reverse mutation test, suggesting that the study drug is not a mutagenic compound.

An increase in frequency of aberrant cells was noted at PROJECT W doses of 400 mcg/mL or more in the in vitro chromosomal aberration test using Chinese hamster lung cells (CHL/IU cells). It was concluded that PROJECT W induced chromosomal aberrations with and without metabolic activation though this was apparent only at the concentrations exhibiting significant cytotoxicity (> 50%).

To determine the relevance of the positive finding in the chromosomal aberration assay, 2 in vivo assays were conducted. An unscheduled DNA synthesis (UDS) test using rat hepatocytes (37.5, 75 and 150 mg/kg PROJECT W orally administered to male rats once)

showed neither increase in net nuclear grain counts or incidence of cells in repair in isolated hepatocytes. The plasma exposure levels (AUC24) at the highest dose in the UDS was 536914.27 ng·h/mL. A micronucleus test using mice bone marrow cells (62.5, 125 and

250 mg/kg PROJECT W orally administered to mice for 2 days) showed no significant increase

in micronucleated polychromatic erythrocytes in femoral bone marrow in any test article group when compared with the negative control group, although in males, but not in females, a statistically significant decrease in polychromatic erythrocytes was noted at doses at or greater than 62.5 mg/kg. The plasma exposure levels (AUC24) at the highest doses in the micronucleus test were 763855.38 and 98543.74 ng·h/mL, in males and females, respectively. The systemic exposures were sufficient to assess the genotoxic potential and it was concluded that the positive results seen in the chromosomal aberration test were irrelevant.

Therefore, it was concluded that PROJECT W has no genotoxic potential.

### Table 5 Genotoxicity Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing Particulars** | **Sex/ No.** | **Doses** | **Major Findings** | **Study No. [Reference]** |
| **Reverse mutation test** | *S. typhimurium*  (TA100, TA1535, TA98, TA1537) | - | 5–5000 mcg/ plate | No increase in revertant colonies | Project W-TX-0005 |
|  | *E. coli* (WP2*uvrA*) |  |  |  |  |
| **Chromosomal aberration test** | CHL/IU cells Treatment for 6 h with or without metabolic  activation system (S9) | - | 100-600 mcg/mL in short-term treatment without  and with | without S9 400-500 mg/kg †: increase in aberrant  cells | Project W-TX-0006 |
|  |  |  | metabolic | with S9 |  |
|  |  |  | activation | 500 mg/kg †: |  |
|  |  |  | 25-200 mcg/mL | increase in aberrant |  |
|  |  |  | in continuous | cells |  |
|  |  |  | treatment for 24 h |  |  |
| **Unscheduled DNA**  **synthesis test** | Rat, SD, oral gavage, single  Liver sampled at 2 and 16 h post-dose | 3M | 0  37.5 mg/kg  75 mg/kg  150 mg/kg | No increase in net nuclear grain counts  or incidence of cells in repair in isolated | Project W-TX-0007 |
|  |  |  |  | hepatocytes |  |
| **Micronucleus test** | Mouse, ICR, oral gavage for 2 days | 5M,  5F | 0  62.5 mg/kg 125 mg/kg | No increase in MNPCE in femoral  bone marrow; | Project W-TX-0008 |
|  |  |  | 250 mg/kg | decrease in PCE at |  |
|  |  |  |  | ≥ 62.5 mg/kg in |  |
|  |  |  |  | males |  |

“-“: not applicable; CHL/IU: Chinese hamster lung cell line; ICR: imprinting control region; MNPCE: multinucleated polychromatic erythrocytes; PCE: polychromatic erythrocytes; SD: Sprague Dawley.

† cytotoxicty is greater than 50%.

### Carcinogenicity

As of January 31, 2012, no studies assessing carcinogenicity of PROJECT W have been conducted.

### Reproductive and Developmental Toxicity

Male and female fertility and early embryonic development to implantation were assessed in rats. Embryo-fetal development was assessed in rats and rabbits.

### Male Fertility

Male rats were given PROJECT W orally at 0, 0.1, 1, 10 and 30 mg/kg per day for 4 weeks before mating, throughout the mating period and up to the day before necropsy (Study Project W-TX-0013).

Sperm examination indicated decreases in sperm counts and sperm motility and an increase in the anomaly index at 1 mg/kg per day or more. A decreased fertility index and increased preimplantation loss index were also recorded at these dose levels. Weight changes in the male reproductive organs and histopathological findings in the testis were the same as in the 4-week rat toxicity study. The NOAEL was judged as 0.1 mg/kg per day.

Full reversibility of the effects on male fertility was confirmed in a separate study (Study Project W-TX-0018).

### Female Fertility and Early Embryonic Development to Implantation

Female rats were given PROJECT W orally at 0, 1, 3, 10 and 30 mg/kg per day from 2 weeks before mating through day 7 of gestation (Study Project W-TX-0014). Corresponding Cmax and AUC are shown in [[Table 8](#_bookmark68)].

Prolonged estrus cycles were noted at 10 and 30 mg/kg per day. The magnitude of prolongation was small and did not affect fertility, early embryonic development or implantation. The NOAEL was judged as 3 mg/kg per day.

Full reversibility of the effects on prolongation of estrus cycle was confirmed in a separate study (Study Project W-TX-0020).

### Embryo-fetal Development

Embryo-fetal development was assessed in rats and rabbits. The corresponding studies are enumerated in [[Table 6](#_bookmark61)] and toxicokinetic data are listed in [[Table 8](#_bookmark68)]. The dose levels were determined based on dose-range-finding studies.

In the rat study (Study Project W-TX-0010), decreased fetal weights and retarded ossification were noted at 30 mg/kg per day. A higher frequency of several minor variations, namely dilated ureter and wavy ribs, was recorded at the same dose and was not considered adverse. Dilation of the ureter and wavy ribs have been associated with reductions in fetal weight.

Suppressed food intake in dams was noted at 10 and 30 mg/kg per day. Body weight gain was suppressed at 30 mg/kg per day. The changes in food intake and in body weight were of statistical significance. The NOAELs in dams and embryo-fetal development were judged as 1 and 10 mg/kg per day, respectively.

In the rabbit study (Study Project W-TX-0012), retarded ossification was noted at 30 and

100 mg/kg per day. Effects on fetal and placental weights were obvious at 100 mg/kg per day. Decreases in dam body weight gain and food intake were noted at 30 and 100 mg/kg per day. No abnormalities were noted in external, visceral or skeletal examinations of embryos. The NOAEL in dams and embryo-fetal development, therefore, was judged as 10 mg/kg per day.

It was concluded that PROJECT W has no teratogenic potential.

### Table 6 Embryo-fetal Development Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing Particulars** | **Sex/ No.** | **Doses (mg/kg)** | **Major Findings** | **Study No.** |
| Fertility and early embryonic development to implantation | Rat, SD, oral gavage | 100M | 0  0.1  1  10  30 |  1 mg/kg: decreases in sperm counts and sperm motility, an increase in sperm anomaly index, decreased fertility index, increased pre- implantation loss index, decreased epididymis weight, spermatid retention  30 mg/kg: decreases in food intake and body weight gain, decreases in prostate and seminal vesicle weight | Project W-TX-0013 |
| Rat, SD, oral gavage | 40M | 0  10 | Reversibility  The findings observed in Project W-TX-0013 fully  disappeared following a  4-week nontreatment recovery period. | Project W-TX-0018 |
| Rat, SD, oral gavage | 100F | 0  1  3  10  30 |  10 mg/kg: prolonged estrus cycles  30 mg/kg: transiently increased locomotor activity/irritability, decreases in food intake and body weight  gain | Project W-TX-0014 |
| Rat, SD, oral gavage | 40F | 0  10 | Reversibility  The findings observed in Project W-TX-0014 fully recovered  following a 2-week nontreatment recovery period | Project W-TX-0020 |
| *Table continued on next page* | | | | | |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain,**  **Dosing Particulars** | **Sex/No.** | **Doses (mg/kg)** | **Major Findings** | **Study No.** |
| **Embryo- fetal developmen t** | Rat, SD, oral gavage dosing during days 7 to 17 of gestation | 6F† | 0  3  10  30 | Dams   10 mg/kg: decreases in body weight gain and food intake  30 mg/kg: increased spontaneous activity | Non-GLP dose range finding study  (Project W-TX- 0009) |
|  |  |  |  | Fetuses |  |
|  |  |  |  |  10 mg/kg: decreased weight |  |
|  |  | 19–20F | 0 | Dams | Project W-TX-0010 |
|  |  |  | **1** (D)  **10** (F)  30 |  10mg/kg: decreases in food intake  30 mg/kg: suppressed body |  |
|  |  |  |  | weight and decreased food |  |
|  |  |  |  | intake |  |
|  |  |  |  | Fetuses |  |
|  |  |  |  | 30mg/kg: decreased fetal |  |
|  |  |  |  | weight, increased placental |  |
|  |  |  |  | weight, higher frequency of |  |
|  |  |  |  | dilated ureter and wavy rib, |  |
|  |  |  |  | retarded ossification |  |
| **Embryo- fetal developmen t** | Rabbit, New Zealand white, oral gavage dosing during days 6 to 18 of gestation | 5–6F | 0  30  100  300 | Dams   30 mg/kg: decreased food intake   100 mg/kg: suppressed body weight  300 mg/kg: moribund sacrifice | Non-GLP dose range finding study  (Project W-TX- 0011) |
|  |  |  |  | Fetuses |  |
|  |  |  |  | No effects |  |
|  |  | 17–20F | 0 | Dams | Project W-TX-0012 |
|  |  |  | **10** (D, F)  30  100 |  30 mg/kg: decreased food intake and suppressed body  weight |  |
|  |  |  |  | Fetuses |  |
|  |  |  |  |  30 mg/kg: retarded |  |
|  |  |  |  | ossification |  |
|  |  |  |  | 100 mg/kg: decreases in fetal |  |
|  |  |  |  | and placental weight |  |

The No Observed Adverse Effect Levels (NOAELs) are bold and underlined, D: NOAEL to the dams, F (in ‘Sex/No’ column): female; F (in ‘Doses’ column): NOAEL to the fetuses; GLP: Good Laboratory Practice; SD: Sprague Dawley.

† One animal in the control group and 2 animals in the 3 mg/kg per day groups were not pregnant

### Local Tolerance

To date, no local tolerance studies have been performed.

### Other Toxicity Studies

### Physical Dependency

Physical dependence was assessed in rats using the abstinence withdrawal procedure [[Table 7](#_bookmark65)]. During a post-dosing period following a 4-week repeated-administration period,

reaction behavior against handling by laboratory workers was exaggerated and body weight gain was suppressed. Diazepam was given as a positive control to males only.

From day 2 of the withdrawal period, male rats exhibited hyper-reactivity to handling at all doses of PROJECT W without a relation to dose. On day 2 of withdrawal, male rats exhibited slightly lower body weight gain at the highest PROJECT W dose compared to vehicle control. No clear withdrawal signs in gross behavior, body weights or food consumption were seen in female rats. The males given diazepam exhibited similar hyper-reactivity to handling but much larger decreases in food consumption and body weight loss from day 2 of withdrawal, indicating development of physical dependence.

The degree of withdrawal signs observed in male rats was mild, and none was observed in female rats, while the positive control, diazepam, induced a clear response in male rats. The findings with PROJECT W were not typical nonclinical observations associated with cannabinoid withdrawal related to abrupt cessation of treatment with a CB1 receptor.

Therefore, the physical dependence potential appears low, but cannot be completely excluded (Study Project W-TX-0016).

### Table 7 Physical Dependency Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing Particulars** | **Sex/No**  **.** | **Doses (mg/kg)** | **Major Findings** | **Study No.** |
| **2 weeks physical dependency (dose range finding)** | Rat, SD,  Oral gavage | 5M, 5F | 0  3  10  30 | Dosing period (2 weeks) 30 mg/kg: prone position, decreases in food intake and body weight  Post-dosing period   3 mg/kg: suppressed | Project W-TX-0015 Non-GLP |
|  |  |  |  | food intake |  |
|  |  |  |  | 10 mg/kg: decreased |  |
|  |  |  |  | body weight |  |
| **4 weeks physical dependency** | Rat, SD,  Oral gavage | 10M,  10F | 0  1  3  10 | Dosing period (4 weeks) No effects  Post-dosing period   1 mg/kg: hyper- | Project W-TX-0016 |
|  |  |  |  | reactivity to handling |  |
|  |  |  |  | 10 mg/kg: suppressed |  |
|  |  |  |  | body weight gain |  |

GLP: Good Laboratory Practice; SD: Sprague Dawley.

### Measurement of Serum Hormone Concentration in Rats

Assessment of effects on hormone levels was conducted in rats (Study Project W-TX-0023). A single oral dose of PROJECT W at 3 or 30 mg/kg led to elevation in adrenocorticotropic hormone levels in both sexes, a decrease in testosterone levels in males and a decrease in luteinizing hormone (LH), follicle-stimulating hormone (FSH) and progesterone levels in females. At 30 mg/kg, low LH, FSH and corticosterone levels were observed in males, while high or low estrogen levels were observed in females.

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

PROJECT W is a selective FAAH-1 inhibitor. FAAH is an enzyme that catalyzes the hydrolysis of ethanolamides (such as AEA). Thus, PROJECT W inhibits the breakdown of ethanolamides thereby increasing their levels. Increased AEA levels have been demonstrated to induce an analgesic effect, which is considered to be mediated by both the CB1 and CB2 receptors although other mechanisms of actions or pathways may also be involved (e.g., PEA, TRPV1 or monoamine efflux).

In vitro, PROJECT W inhibited human FAAH-1, rat FAAH and monkey FAAH-1 activities with IC50 values of 3.99, 4.42 and 34.5 nM, respectively, in FAAH-1 expressing COS-7 cells.

PROJECT W at 10 mcmol/L did not significantly interact with 64 different kinds of receptors, ion channels, transporters and enzymes, including cannabinoid receptors.

Orally administered PROJECT W induced a significant analgesic effect in in vivo models of NP and OA pain at a dose range of 0.3 to 3 mg/kg. Since acute administration of PROJECT W at 10 mg/kg or more resulted in cannabinoid-related adverse effects, the true active dose range is judged to be 0.3 to 3 mg/kg in rats.

Tolerance or sensitization to the pharmacological effect did not occur, as demonstrated in a 4-week, repeat-dose, pharmacology study that examined mechanical allodynia in the SNL

model. Also, the analgesic effect was long-lasting and persisted for 24 hours following acute dosing in models of NP. This prolonged analgesic effect likely cannot be explained by the pharmacokinetic profile or by increased AEA levels, since brain AEA levels were reduced to basal values 24 hours after acute administration of PROJECT W. However, it remains possible that AEA levels were increased for longer periods of time during states of pain since the experiment examining the effect of PROJECT W on AEA levels was conducted under nonpainful conditions.

Although neither tolerance nor sensitization developed in the 4-week, repeat-dose, pharmacology study, a toxicology study indicated that, following abrupt discontinuation after 4 weeks of treatment, there were some withdrawal signs, namely hyperreactivity and suppressed body weight. These signs were mild compared to the positive control, diazepam, and strikingly different from the typical CB1-related withdrawal signs, such as wet-dog shakes, facial rubbing, tremor, ptosis, and scratching. Altogether, the dependence potential of PROJECT W appears low, but cannot be excluded based on the current data.

Plasma drug concentrations increased more than dose proportionally from 0.3 to 3 mg/kg oral administration. This more than dose-proportional increase was also confirmed in the

dose-range of the toxicity studies (0.1 to 30 mg/kg oral administration) [[Table 8](#_bookmark68)]. The drug showed good oral bioavailability. In addition, PROJECT W easily penetrated the brain and the drug concentration ratio of brain tissue to plasma was in the range of 0.44 to 0.62. In spite of its lipophilic profile, it was confirmed in radioactivity studies that there is no concern of

long-term drug retention in tissues. An in vitro assay showed that the plasma protein binding ratio varied between animals and humans (73.4%, 74.3% and 85.1% for rats, monkeys and humans, respectively). Furthermore, there were no human specific metabolites detected in an in vitro assay. An in vitro CYP assay showed inhibitory potentials on CYP2C8, 2C9, 2C19, 2D6 and 3A4/5. The absorbed drug in rats was mainly excreted through the kidney.

The major findings in the toxicity studies were sedation, lowered BP, lowered body temperatures, impaired male fertility (spermatid retention) and prolonged estrus cycles. Nonclinical toxicology findings indicate that PROJECT W has no teratogenic or genotoxic potential.

All toxicology findings occurred at a dose of 10 mg/kg per day or more with the exception of spermatid retention, which occurred at 0.3 mg/kg per day. Therefore, the effect on male fertility overlaps with the active dose range while the other adverse effects do not.

The underlying mechanisms of the toxicology findings have not yet been fully investigated. With the exception of the spermatid retention in rats, the observed toxicology findings are similar to those anticipated for directly acting cannabinoid agonists; thus, it is currently believed that all toxicity findings are mediated by modulation of activity at the endocannabinoid system. All toxicity findings are monitorable in a clinical setting.

In conclusion, PROJECT W was efficacious across a range of nonclinical models of pain. Efficacy was less robust in models of inflammatory pain, and acute nociceptive pain was not affected (preliminary data). Robust signs of efficacy were seen in models of neuropathic and OA pain. Consequently, PROJECT W is being explored for these indications. The effect in pharmacological models was not affected by confounding factors on behavior at the dose range of 0.3 to 3.0 mg/kg. Most adverse effects occurred at doses that were above the pharmacologically active dose range and were relatively mild in nature. However, an adverse effect on sperm function occurred well within the pharmacologically active dose range but was found to be reversible. Results of the completed clinical studies show no evidence of a unique human metabolite.

### Table 8 Exposure Ratios of PROJECT W Between Animals and Humans

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species/Study Duration** | **Dose (mg/kg)** | **Sex (M/F)** | **AUC24h**  **(ng****h/mL)** | **Exposure Ratios† Based on 30 mg BID Human Dose** |
| **Rat, 13 Weeks, PO (Project W-TX-0021)** | 0.1  (NOAEL) | M | 13.8 | 0.0 |
| 0.3  (LOAEL) | M | 123 | 0.0 |
| 3  (NOAEL) | F | 9512 | 0.7 |
| 30  (LOAEL) | F | 280741 | 21.2 |
| **Monkey, 13 Weeks, PO (Project W-TX-0022)** | 5  (NOAEL) | M | 14250 | 1.1 |
| F | 13832 | 1.0 |
| 30  (LOAEL) | M | 279824 | 21.1 |
| F | 219419 | 16.6 |
| **Human, Healthy Subjects, 10 Days, PO (Project W-CL-0002)** | 30 mg bid | F | 13256 | NA |

AUC: area under the curve; LOAEL: lowest observed adverse effect level; NA: not applicable; NOAEL: no observed adverse effect level.

†Exposure ratios were calculated by dividing the AUC24h in animals by the 2-fold AUCtau (6628 ng·h/mL) in healthy postmenopausal females at the maximum target dose of 30 mg bid in the phase 2A study.