### Safety Pharmacology

### Central Nervous System

There were no effects of Project 19 observed in the central nervous system in rats (Study Project 19-PT-0004).

### Cardiovascular System

Project 19 did not affect in vitro action potential duration in guinea pig papillary muscle and human cardiac potassium channel (human ether-a-go-go-related gene [hERG] assay) currents at concentrations up to 10 mcM (Studies Project 19-PT-0001 and Project 19-PT-0002). In an in vivo safety pharmacology study in conscious cynomolgus monkeys, no effects were noted on the cardiovascular system at doses up to 1000 mg/kg (Study Project 19-PT-0003).

### Respiratory System

There were no effects of Project 19 observed in the respiratory system in cynomolgus monkeys (Study Project 19-PT-0003).

### Pharmacodynamic Drug Interactions

Key findings from the studies of in vivo pharmacodynamic drug interaction are presented in [Table 4.](#_bookmark19)

### Table 4 Summary of Pharmacodynamic Drug Interaction Studies Conducted with Project 19 In Vivo

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study No.** | **Species, Sex, No.** | **Dosage, Route, Treatment Duration** | **Endpoint(s)** | **Major Findings** |
| **Project 19-PH-0010**  **Project 19-PH-0011** | Normal ICR mice, male (n=4) | Single administration  Glibenclamide: 3 mg/kg po  Combination: glibenclamide (3 mg/kg p.o) and Project 19 (0.03, 0.1, 0.3, 1, 3, 10,  30 mg/kg po) | Blood glucose AUC for 6 h in OGTT and fasted condition | The combination showed additive effects on reducing blood glucose levels in OGTT and fasted condition.  Add-on effects of Project 19 on blood glucose were more effective in OGTT with significance at ≥0.1 mg/kg than in fasted condition with significance at ≥10 mg/kg. |
| **Project 19-PH-0022** | KK-Ay  mice, male (n=8) | Repeated administration for 4 weeks  Project 19: 0.3 mg/kg po, once daily Metformin: 100 mg/kg po, twice daily  Combination of the above | HbA1c | The combination further reduced HbA1c levels when compared with monotherapy with Project 19 or metformin. |
| **Project 19-PH-0026** | KK-Ay  mice, male (n=5) | Single administration  Project 19: 0.3, 1, 3,  10, 30 mg/kg po  Metformin: 200 mg/kg po | Fasting blood glucose | Compared with Project 19 monotherapy, the combined treatment slightly increased the number of animals with fasting blood glucose levels <70 mg/dL. |
|  |  | Combination of the above |  |  |
|  |  | Repeated administration for 4 weeks |  |  |
|  |  | Project 19: 0.3, 1, 3,  10, 30 mg/kg po, once daily  Metformin: 200 mg/kg po, twice daily |  |  |
|  |  | Combination of the above |  |  |
| **Project 19-PH-0023** | KK-Ay  mice, male (n=8) | Repeated administration for 4 weeks  Project 19: 0.3 mg/kg po, once daily Pioglitazone: 10 mg/kg po, once daily | HbA1c | The combination further reduced HbA1c levels when compared with monotherapy with Project 19 or pioglitazone. |
|  |  | Combination of the above |  |  |

### [Table 4](#_bookmark19) continued

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study No.** | **Species, Sex, No.** | **Dosage, Route, Treatment Duration** | **Endpoint(s)** | **Major Findings** |
| **Project 19-PH-0024** | KK-Ay  mice, male (n=8) | Single administration  Project 19: 0.3 mg/kg po  Voglibose: 0.3 mg/kg po | Incremental blood glucose AUC for 2 h after liquid meal loading | The combination further reduced hyperglycemia after liquid meal loading when compared with monotherapy with Project 19 or voglibose. |
|  |  | Combination of the above |  |  |
| **Project 19-PH-0028** | Normal ICR mice, male (n=10) | Single administration  Project 19: 0.3 mg.kg po  Sitagliptin: 1 mg/kg po | Blood glucose AUC for 2 h after liquid meal loading | The combination further reduced hyperglycemia after liquid meal loading when compared with monotherapy with Project 19 or sitagliptin. |
|  |  | Combination of the above |  |  |
| **Project 19-PH-0035** | Normal ICR mice, male (n=8) | Single administration  Project 19: 0.3 mg/kg po  Nateglinide: 25 mg/kg po | Blood glucose AUC for 2 h after glucose loading | The combination further reduced hyperglycemia after glucose loading when compared with monotherapy with Project 19 or nateglinide. |
|  |  | Combination of the above |  |  |

EOTF: End of Text Figure; EOTT: End of Text Table.

Source: Studies Project 19-PH-0010, Project 19-PH-0011, Project 19-PH-0022, Project 19-PH-0023, Project 19-PH-0024, Project 19-PH-0026, Project 19-PH-0028, Project 19-PH-0035

### Other Pharmacology Studies

Key findings from another pharmacology study are presented in [Table 5.](#_bookmark21)

### Table 5 Summary of Other Pharmacology Studies Conducted with Project 19 In Vivo

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study No.** | **Species, Sex, No.** | **Dosage, Route, Treatment Duration** | **Endpoint(s)** | **Major Findings** |
| **Project 19-PH-0031** | Diet-induced obesity rat, Male (n=7 or  8) | Repeated administration Project 19: 1, 3,  10 mg/kg po | Body weight, body weight gain and epididymal  fat mass | Project 19 reduced body weight, body weight gain and fat mass at  10 mg/kg |

EOTF: End of Text Figure; po: oral administration. Source: Study Project 19-PH-0031

## Toxicology

### Single-dose Toxicity

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

Project 19 at doses of up to 2000 mg/kg was tolerated in male rats, but doses of 1000 and 2000 mg/kg were lethal for female rats. Gastric mucosal damage was recorded at postmortem examination.

### Single Oral Dose Toxicity Study in Monkeys

A dose of up to 2000 mg/kg was tolerated in both sexes. Vomiting, soft stool and a decrease in food consumption were observed at 1000 mg/kg or more.

### Table 6 Toxicokinetics in a Single Dose Toxicity Study of Project 19 in Monkeys

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species, Strain** | **Dose† (mg/kg)** | **No. Sex (M/F)** | **Cmax (ng/mL)** | **AUC24**  **(ng•h/mL)** |
| **Cynomolgus monkey** | 1000 | 1 M  1 F | 93800  74700 | 1340000  1190000 |
| 2000 | 1 M  1 F | 109000  109000 | 2230000  1260000 |

† Single dose, oral gavage in 0.5% methylcellulose. Source: Study Project 19-TX-0005

### Repeat-dose Toxicity

Repeated dose toxicity was assessed in rats and monkeys up to 26 weeks and 52 weeks, respectively.

### Repeated Oral Dose Toxicity Studies in Rats

In a 2-week study, Sprague-Dawley rats were given doses of 0, 1, 10, 100 and

1000 mg/kg/day (Study Project 19-TX-0006). In 13-week and 26-week studies, doses of 0, 0.1, 1, 10 and 100 mg/kg/day were given to Sprague-Dawley rats (Studies Project 19-TX-0015 and Project 19- TX-0021). A dose of 1000 mg/kg/day could not be tolerated and 1 male rat died on Day 6.

UGE increased dose dependently, which was the primary pharmacological effect. Urine volume, water intake, electrolyte excretion and kidney weight increased at doses of

1 mg/kg/day or more. These effects were considered to be attributable to exaggerated pharmacology and caused by glucosuria-related osmotic diuresis. In addition, despite increased food intake, body weight gain was suppressed. Glycogen deposition in renal tubules and enlargement of vacuoles in brown adipose tissue were recorded by microscopy. These findings were also considered to be related to excessive glucose excretion into urine.

A statistically significant decrease in plasma glucose levels was observed at 10 mg/kg/day or more. No symptoms indicating hypoglycemia were recorded.

At 1 mg/kg/day or more, urinary NAG and beta-2-MIC excretion increased.

At 10 and 100 mg/kg/day, blood tests showed increases in BUN and liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]). Histopathology did not detect any degenerative changes in the liver tissue. Renal histopathology showed dilatation of renal tubules and hypertrophy of the tubular epithelium. Degenerative changes were not detected in the renal tissues. In the gastrointestinal tract, mucosal damage was detected in the stomach and duodenum.

In addition, blood tests indicated low values for erythrocyte counts, hematocrit, hemoglobin, globulin and albumin at 10 mg/kg.

Reversibility was confirmed for all findings.

Hypertrophy in the adrenal cortex was noted only in the 2-week rat study at 1 mg/kg/day or more. This histological finding was minimal or mild, and reversible. None of the other repeated dose toxicity studies in rats or monkeys reported histological alterations in the adrenal tissue. This hypertrophy in the adrenal cortex in the 2-week rat study was therefore considered to be toxicologically insignificant.

Since urinary NAG and beta-2-MIC excretion increased at 1 mg/kg/day, the no observed adverse effect level (NOAEL) was judged to be 0.1 mg/kg/day.

Cmax and AUC values increased almost dose-proportionally in both sexes. Cmax and AUC values were slightly higher in females than those in males [[Table 7](#_bookmark60)]. The tmax values did not obviously differ between doses or sexes. There were no changes in the pharmacokinetic parameters following repeated administration compared with the first dose.

### Table 7 Toxicokinetics in Repeated Dose Toxicity Studies of Project 19 in Sprague Dawley Rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Dosing particulars** | **No.** | **Sex (M/F)** | **Daily Dose**  **(mg/kg)** | **Cmax (ng/mL)** | | **AUC24 (ng•h/mL)** | | **Study No.** |
| **First dose** | **Last dose** | **First dose** | **Last dose** |
| **2 weeks (Oral gavage in 0.5% MC)** | 3 | M | 1 | 247 | 245 | 1730 | 1550 | Project 19-TX-0006 |
| 3 | F | 398 | 540 | 2610 | 2760 |
| 3 | M | 10 | 2500 | 2260 | 15200 | 13500 |
| 3 | F | 4280 | 5050 | 35800 | 27200 |
| 3 | M | 100 | 21600 | 19900 | 219000 | 170000 |
| 3 | F | 36900 | 33700 | 418000 | 275000 |
| 3 | M | 1000 | 73300 | 135000 | 1570000 | 1820000 |
| 3 | F | 123000 | 144000 | 2190000 | 2250000 |
| **13 weeks (Oral gavage in 0.5% MC)** | 3 | M | 0.1 | 21.3 | 21.6 | 198 | 139 | Project 19-TX-0015 |
| 3 | F | 40.0 | 48.3 | 347 | 310 |
| 3 | M | 1 | 174 | 217 | 1590 | 1350 |
| 3 | F | 332 | 590 | 3460 | 3350 |
| 3 | M | 10 | 2200 | 2390 | 18500 | 15200 |
| 3 | F | 4120 | 5980 | 35000 | 40300 |
| 3 | M | 100 | 23200 | 16500 | 245000 | 177000 |
| 3 | F | 32400 | 30100 | 444000 | 386000 |
| **26 weeks (Oral gavage in 0.5% MC)** | 3 | M | 0.1 | 13.8 | 27.5 | 131 | 175 | Project 19-TX-0021 |
| 3 | F | 23.5 | 42.4 | 263 | 432 |
| 3 | M | 1 | 156 | 333 | 1380 | 1460 |
| 3 | F | 319 | 560 | 2500 | 4370 |
| 3 | M | 10 | 1690 | 3250 | 15700 | 19000 |
| 3 | F | 3030 | 5590 | 27100 | 37500 |
| 3 | M | 100 | 16300 | 14700 | 133000 | 139000 |
| 3 | F | 30600 | 35800 | 305000 | 508000 |

MC: Methylcellulose.

Source: Studies Project 19-TX-0006, Project 19-TX-0015, Project 19-TX-0021

### Repeated Oral Dose Toxicity Studies in Monkeys

In a 2-week study, cynomolgus monkeys were given doses of 0, 10, 100 and 1000 mg/kg/day (Study Project 19-TX-0007). One female administered 1000 mg/kg/day died on Day 10.

In a 13-week study, cynomolgus monkeys were given doses of 0, 10, 100 and 300 mg/kg/day (Study Project 19-TX-0014). In a 52-week study, cynomolgus monkeys were dosed at 0, 1, 10 and 300 mg/kg/day (Study Project 19-TX-0022). Project 19 was tolerated up to 300 mg/kg/day.

Pharmacology-related UGE was recorded in all treatment groups.

In the 2- and 13-week toxicity studies in cynomolgus monkeys, animals showed no decreases in plasma glucose levels. In the 52-week study, 1 female at 10 and 300 mg/kg/day doses showed decreased plasma glucose levels though the changes were marginal and no symptoms indicating hypoglycemia were observed.

At 100 mg/kg or more, urinary NAG and beta-2-MIC excretion increased. Histopathology examination did not detect any abnormalities in the renal tissues. In the 52-week study, 1 female at 10 mg/kg/day showed slight increases in urinary NAG and beta-2-MIC excretion. Individual data indicated that a limited number of monkeys showed changes in blood triglyceride, urinary ketone body, BUN and blood liver enzyme at limited time points. All of these changes were marginal and reversible.

The drug exposure increased in a dose dependent manner over the dose range of 1 to

100 mg/kg/day, while it increased less dose-proportionally towards 1000 mg/kg/day. Cmax and AUC at Week 26 slightly increased compared with those on Day 1. The systemic exposures were unchanged thereafter. Sex differences were not apparent [[Table 8](#_bookmark62)].

Although there was no sex difference in terms of exposure, the NOAEL in the 52-week study was judged to be 10 mg/kg/day for males and 1 mg/kg/day for females because 1 female at 10 mg/kg/day showed slight increases in urinary NAG and beta-2-MIC excretion.

### Table 8 Toxicokinetics in Repeated Dose Toxicity Studies of Project 19 in Cynomolgus Monkeys

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Dosing particulars** | **No.** | **Sex (M/F)** | **Daily Dose**  **(mg/kg)** | **Cmax (ng/mL)** | | **AUC24 (ng•h/mL)** | | **Study No.** |
| **First dose** | **Last dose** | **First dose** | **Last dose** |
| **2 weeks (Oral gavage in 0.5% MC)** | 3 | M | 10 | 3330 | 4240 | 25800 | 32000 | Project 19-TX-0007 |
| 3 | F | 3650 | 3350 | 28000 | 33500 |
| 3 | M | 100 | 29500 | 40600 | 269000 | 315000 |
| 3 | F | 39300 | 32800 | 375000 | 341000 |
| 6 | M | 1000 | 88000 | 121000 | 1290000 | 1550000 |
| 6 (5) | F | 76000 | 103000\* | 1050000 | 1230000\* |
| **13 weeks (Oral gavage in 0.5% MC)** | 3 | M | 10 | 3400 | 5610 | 28700 | 33500 | Project 19-TX-0014 |
| 3 | F | 3570 | 3630 | 31600 | 29300 |
| 3 | M | 100 | 22100 | 31100 | 251000 | 240000 |
| 3 | F | 30900 | 27100 | 309000 | 279000 |
| 6 | M | 300 | 41900 | 48200 | 546000 | 520000 |
| 6 | F | 38700 | 49500 | 489000 | 448000 |
| **52 weeks (Oral gavage in 0.5% MC)** | 4 | M | 1 | 344 | 546 | 2430 | 3880 | Project 19-TX-0022 |
| 4 | F | 435 | 608 | 2300 | 3780 |
| 4 | M | 10 | 3120 | 4980 | 24600 | 46200 |
| 4 | F | 3930 | 4050 | 27200 | 34800 |
| 4 | M | 300 | 32500 | 53800 | 508000 | 804000 |
| 4 | F | 35500 | 75000 | 436000 | 903000 |

MC: Methylcellulose.

\* Values after the last dose were obtained from 5 females because 1 female died on Day 10 of dosing. Source: Studies Project 19-TX-0007, Project 19-TX-0014, Project 19-TX-0022

### Genotoxicity

A bacterial reverse mutation test (Study Project 19-TX-0008), a chromosomal aberration test with cultured hamster lung cells (Study Project 19-TX-0009), a micronucleus test in the bone marrow cells obtained from the femur of rats (2000 mg/kg in males and 1000 mg/kg in females) (Study Project 19-TX-0013), and an unscheduled deoxyribonucleic acid (DNA) synthesis assay (Study Project 19-TX-0030) were conducted. Taken collectively, it was concluded that Project 19 has no genotoxic potential.

### Carcinogenicity

### 104-week Carcinogenicity Study in Mice

In a 2-week preliminary study in mice (0, 500, 1000 and 2000 mg/kg/day), no lethal dose level was established (Study Project 19-TX-0033). However, since the maximum feasible concentration was 100 mg/mL and a dose volume of 10 mL/kg/day was considered the maximum feasible dose volume for 13 weeks, the highest dose level of 1000 mg/kg/day was selected in the next dose-range finding study.

In a 13-week dose-range finding study in mice (0, 250, 500 and 1000 mg/kg/day), a dose level of 1000 mg/kg/day induced more than a 10% increase in body weight with increased food consumption, clear toxicity in the kidney, liver, and bones, including vacuolation of the proximal tubular cells and dilatation of the distal/collecting tubules in the kidney, eosinophilic changes in the hepatocytes, and increased trabecular bone in the femur (Study Project 19-TX-0035).

In a definitive 104-week carcinogenicity study in mice (0, 50, 150 and 500 mg/kg/day), there were no treatment-related increases in the incidence of any tumor in either sex (Study Project 19- TX-0024).

### 104-week Carcinogenicity Study in Rats

In a 2-week preliminary study in rats (0, 500, 1000 and 2000 mg/kg/day), deaths in males and females were noted at 2000 mg/kg/day (Study Project 19-TX-0034).

In a 13-week dose-range finding study in rats (0, 250, 500 and 1000 mg/kg/day), males administered 250 mg/kg/day and females administered 500 mg/kg/day showed a decrease in body weight of more than 10% relative to the control animals. The highest dose levels were set at 125 mg/kg/day for males and 250 mg/kg/day for females, respectively, as the maximum tolerated dose for the carcinogenicity study (Study Project 19-TX-0036).

In a definitive 104-week carcinogenicity study in rats (0, 12.5, 40, 125 and 250 [only females] mg/kg/day), histopathological examination revealed increased incidences of pheochromocytomas in the adrenal in males at 40 and 125 mg/kg/day and in females at 125 and 250 mg/kg/day. An increased combined incidence of medullary hyperplasia and pheochromocytomas in the adrenal was observed in males at 12.5 mg/kg/day or more and in females at 40 mg/kg/day or more. Incidences of all other tumors were comparable to those in controls (Study Project 19-TX-0025) [[Table 9](#_bookmark67)].

### Table 9 Summary of Neoplastic Changes and Tumor-related Proliferative Changes

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sex  Dose (mg/kg/day) | 0 | Male  12.5 40 | | 125 | 0 | Female  12.5 40 | | 125 | 250 a) |
| No. of animals used | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Adrenal |  |  |  |  |  |  |  |  |  |
| Hyperplasia, medullary, |  |  |  |  |  |  |  |  |  |
| Focal |  |  |  |  |  |  |  |  |  |
| ± | 16 | 18 | 14 | 8 | 8 | 16 | 11 | 5 | 1 |
| + | 14 | 20 | 24 | 23 | 4 | 11 | 37 | 32 | 36 |
| ++ | 1 | 4 | 9 | 14 | 0 | 0 | 3 | 4 | 6 |
| Pheochromocytoma, 9 17 22 ## 36 ## 4 | | | | | | 3 | 5 24 ## 29 ## | | |
| Pheochromocytoma, 0 | | 2 | 1 | 2 | 1 | 0 | 0 | 0 | 0 |
| Hyperplasia, medullary, Focal + |  |  |  |  |  |  |  |  |  |
| Pheochromocytoma, Benign + 34 50 ## | | | 55 ## | 54 ## 16 28 52## 52 ## 55 ## | | | | | |



Benign Malignant

Pheochromocytoma, Malignant



##: p≤0.01 (statistically significant difference for positive between control group and dose groups, Peto’s exact test)

±: minimal; +: mild; ++: moderate

* + - * 1. : At Week 99 just after administration was discontinued.

In addition, histopathological examination revealed increased incidence and/or severity of mineralization (calcification) in the kidney, cornea in the eye, arterial wall in the heart, lung and tongue and increased incidence and severity of hyperostosis in the sternum and femur. Furthermore, increases in plasma calcium and inorganic phosphorus concentrations were observed in a 13-week dose-range finding study.

It is known that there are general differences in the function of the adrenal medulla between rats and other species including humans and that the incidence of proliferative adrenal medullary lesions increases in rats with the intake of various chemical substances, i.e., the food additives polyols (mannitol, xylitol, etc.), lactose, or vitamin D [Tischler AS et al., 1996]. Increased calcium absorption due to ingestion of polyols, lactose, and vitamin D has been explained as the cause of adrenal medullary tumors in rats [Roe FJC, 1990]. Since adrenal pheochromocytomas do not occur in mice, dogs, or humans even with long-term administration of these substances, the proliferative adrenal medullary lesions in rats given polyols or lactose are considered a species-specific phenomenon unique to rats [WHO Technical Report Series, 1997].

The mechanism by which Project 19 causes adrenal medullary tumors and the risks to humans are thought to be as follows. In rats administered high doses of at least 125-fold the effective dose (0.1 mg/kg) in the rat carcinogenicity study, increased food intake persisted throughout treatment. This is compensation for excessive urinary glucose excretion caused by excessive SGLT2 inhibition. It is suspected that excessive intake of calcium and vitamin D due to increased food intake caused increased calcium absorption from the gut and increased catecholamine synthesis in the adrenal medullary cells. There was also a trend toward elevated blood calcium and inorganic phosphorus, as well as systemic metastatic calcium deposition and hyperostosis, suggesting an imbalance in calcium and phosphorus metabolism.

The above findings further suggest that the frequency of adrenal medullary hyperplasia or pheochromocytoma (which occurs spontaneously in rats) increased because of prolonged adrenal medullary cell hyperactivity induced by increased calcium intake [Roe FJC, 1988]. It is also possible that intestinal flora are activated by inhibition of glucose absorption from the gut due to SGLT1 inhibition, and that the resulting lowered pH contributes to increased calcium absorption [Summan M et al., 2011]. Human adrenal medullary cells are less sensitive to calcium than those of rats, and there are no reports of tumors in humans caused by drugs that induce adrenal medullary tumors in rats by the mechanism described above.

Consequently, the pheochromocytoma seen with Project 19 in rats cannot be extrapolated to humans, and it is considered that Project 19 poses no risk of causing development of pheochromocytoma in the adrenal medulla in humans [External Assessment Report Project 19- TX-0039 and Project 19-TX-0040].

### Reproductive and Developmental Toxicity

* + - 1. **Effects on Fertility and Early Embryonic Development to Implantation in Rat**

No effects on reproductive function in male and female rats (estrous cycle, copulation index, fertility index, the days until copulation) or early embryonic development (number of live fetuses, number of embryo-fetal deaths, post-implantation loss rate) were noted (Study Project 19- TX-0016). Because of a high frequency of dead or moribund sacrificed animals (12 males and 16 females) at 1000 mg/kg/day, administration was discontinued on Day 8 of dosing and reproductive performance and early embryonic development could not be investigated at this dose level. At 300 mg/kg/day or more, general toxicological effects such as death, decreased spontaneous motility, moist fur around the urethral orifice, soiled fur around the nose and anus, reddish fur around eyes, lacrimation, loss of hair, reddish urine, wasting, prone position and decreases in body weight and food consumption were noted in male and female rats. At 100 mg/kg/day, body weight gain was decreased in male rats. Increased food consumption observed at 100 mg/kg/day or more was probably related to pharmacological action rather than toxicity.

The general toxicological NOAEL was judged to be less than 100 mg/kg for male animals, and 100 mg/kg/day for female animals. The NOAELs for reproductive performance and early embryonic development were both judged to be 300 mg/kg/day.

### Effects on Embryo-Fetal Development in Rats

In this preliminary study in rats (0, 100, 300 and 1000 mg/kg/day), decreases in fetal and placental weights were noted at the dose of 1000 mg/kg/day (Study Project 19-TX-0010). In dams, 1 animal administered 1000 mg/kg/day exhibited a decrease in spontaneous movement,

ptosis and emaciation, and died on Day 11 of gestation. Survivors at 1000 mg/kg/day showed decreases in body weight and food consumption. Increased food consumption observed at 100 mg/kg/day or more was probably related to the pharmacological action rather than toxicity.

In a definitive GLP study in rats (0, 100, 300 and 600 mg/kg/day), Project 19 was revealed to have no teratogenic or lethal potential in fetuses (Study Project 19-TX-0017). In dams, 3

animals at 600 mg/kg/day died on Days 12 or 18 of gestation. The findings observed in these dead dams included decreased spontaneous motility and moist fur around urethral orifice. In the surviving animals receiving doses of 300 mg/kg/day or more, decreased body weight and food consumptions were seen. There was no effect on the number of corpora lutea or number of implantations at doses up to 600 mg/kg/day. Increased food consumption observed at

100 mg/kg/day or more was probably related to the pharmacological action rather than toxicity. At 600 mg/kg/day, decreases in fetal and placental weights and increased incidence of thymic remnant in the neck in fetuses were observed, indicating a fetal growth retardation effect. There were no changes attributable to the test article in number of live fetuses, post- implantation loss or sex ratio, and in the incidence of fetuses with external and skeletal abnormalities.

Thus, the NOAEL was judged to be 100 mg/kg/day for maternal toxicity and 300 mg/kg/day for embryo-fetal development.

### Effects on Embryo-Fetal Development in Rabbits

In a preliminary study in rabbits (0, 100, 300 and 1000 mg/kg/day), 2 dams treated with Project 19 at the dose of 1000 mg/kg/day died on Days 10 and 11 of gestation (Study Project 19- TX-0011). The 4 surviving dams were sacrificed due to moribund condition on Day 11 of gestation; only dead embryos were observed in the uteri of these dams. At 300 mg/kg/day, dams showed suppressed body weight gain and low food consumption.

In a definitive GLP study in rabbits (0, 30, 100 and 300 mg/kg/day), no effects on

embryo-fetal development, including teratogenic, fetal lethality, and fetal growth retardation effects, were observed (Study Project 19-TX-0032). In dams, general toxicological effects were observed only at 300 mg/kg/day and consisted of the death of 3 dams (Day 23 or 25 of gestation). Changes observed were dark reddish watery discharge, decreased fecal output, suppression of body weight gain and food consumption. After exhibiting these toxicities at 300 mg/kg/day/day, 5 dams aborted between Days 20 and 25 of gestation. Macroscopic observation of deceased or aborting dams exhibited discoloration of various organs and tissues and retention of fur balls. Increased food consumption observed at 30 mg/kg/day or more was probably related to the pharmacological action rather than toxicity.

Thus the NOAEL was judged to be 100 mg/kg/day for maternal toxicity and 300 mg/kg/day for embryo-fetal development.

### Effects on Pre- and Postnatal Development, Including Maternal Function in Rats

The study (0, 30, 100 and 300 mg/kg/day) was conducted to investigate the effects on pregnant and lactating females and on the development of the conceptuses and pups when administered orally to pregnant rats during the period from implantation to weaning (Study Project 19-TX-0023).

In dams, 2 dams died on Day 20 of gestation or Day 1 after delivery (Day 1 of lactation), and total litter loss occurred in 2 dams on Day 0 or 5 after delivery in the 300 mg/kg group.

These dams showed a decrease in spontaneous activity, reddish eye mucus, emaciation, and

severe decreases in body weight and food consumption from a few days before maternal death or total litter loss occurred. No abnormalities were noted in gross pathology in any dam including animals which died and had total litter loss. No test article-related changes were noted in delivery data, except for the above-mentioned total litter loss, in the 300 mg/kg group.

In F1 animals, birth index, viability index on Day 0 after birth, and weaning index tended to be lower at 300 mg/kg/day compared to the control group. Low body weight was noted on Days 14 and 22 after birth in males and females in this group; however, their body weight recovered after weaning in both sexes.

Thus the NOAEL was judged to be 100 mg/kg/day for F0 dams, reproductive function of F0 dams and F1 animals.

### Local Tolerance

### Eye Irritation Test in Rabbits

In an eye irritation test in rabbits, Project 19 was considered mildly irritating. The finding disappeared after 8 days of observation (Study Project 19-TX-0012). This effect in an eye-washed group disappeared at an earlier time compared with that of an eye-unwashed group.

### Local Irritation Test in Rabbits

The venous and perivascular irritancy of Project 19 injection (0.05 mg/mL as Project 19) was evaluated when administered once into or around the auricular veins of rabbits (Study Project 19-TX-0042). It was concluded that Project 19 injection had no venous or perivascular irritancy to rabbits.

### Other Toxicity Studies

* + - 1. **Skin Sensitization Test in Guinea Pigs**

The skin sensitization potential of Project 19 was evaluated in a maximization test in guinea pigs (Study Project 19-TX-0029). It was concluded that Project 19 was a non-skin sensitizer.

### Effects of SGLT2 Inhibitors on Urinalysis Parameters in Rats

In order to evaluate the effects of Project 19 on urinalysis parameters, Project 19 was administered orally to rats once daily for 1 week (Study 543-TX-023). Two other SGLT2 inhibitors, YM543 (a different development compound) and YM-9608557 (i.e., dapagliflozin), were included in this study to examine the class effects of SGLT2 inhibitors on urinalysis parameters in the rat. A preliminary study was performed prior to this study as a dose-range finding study (Study 543-TX-022).

After repeated administration of Project 19 (10 mg/kg/day), YM543 (30 mg/kg/day), and dapagliflozin (1 mg/kg/day), increases in urine volume, urinary glucose concentration, UGE and creatinine clearance values were noted. Project 19 caused statistically significant increases in creatinine-corrected values of NAG and kidney weight; findings that were also

noted for the other two SGLT2 inhibitors. However, histopathology did not show any test article-related changes in the kidney in any of the test article groups.

### Effects of Dapagliflozin and Comparison with Project 19 in Rats

A 4-week repeated dose toxicity study of SGLT2 inhibitors in rats was performed. In the study dapagliflozin was selected as a reference compound (Study Project 19-TX-0020).

It was established that there were clear dose-response curves for UGE and urinary volume for both dapagliflozin and Project 19 [[Table 10](#_bookmark80)]. The range of UGE and urinary volume for dapagliflozin was comparable to that for Project 19. Dose dependent exposure was confirmed for both compounds [[Table 11](#_bookmark81)].

Dapaglifozin groups showed suppressed body weight gain, increased food intake, increases in water intake and urine volume, increases in NAG, beta-2-MIC and electrolyte excretion in urine, high BUN, increased kidney weight, dilatation and hypertrophy of renal tubules, gastric mucosal damage, high values of ALT and AST, and low hematocrit values.

This data demonstrated that dapagliflozin and Project 19 have a comparable toxicity profile in rats.

### Table 10 Daily Urinary Glucose and Urinary Volume in 4-Week Repeated Dose Toxicity Study of Dapagliflozin and Project 19 in Rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **No.** | **Sex** | **Daily Dose (mg/kg)** | **Daily Urinary Glucose (mg)** | | **Daily Urinary Volume (mL)** | | **Study No.** |
| **Day 1** | **Day 26** | **Day 1** | **Day 26** |
| **Control\*** | 10 | M | 0 | 1.7 | 4.6 | 13.6 | 17.5 | Project 19-TX-  0020 |
| **Dapagliflozin** | 10 | M | 0.1 | 917 | 1660 | 20.8 | 30.9 |
| 10 | M | 1 | 2157 | 4381 | 31.9 | 47.0 |
| 10 | M | 10 | 3237 | 7213 | 46.5 | 70.6 |
| 10 | M | 100 | 3677 | 8223 | 45.4 | 72.5 |
| **Project 19** | 10 | M | 1 | 658 | 1028 | 21.5 | 25.9 |
| 10 | M | 10 | 2060 | 4138 | 31.1 | 45.3 |
| 10 | M | 100 | 3379 | 6644 | 47.1 | 60.8 |

\* 0.5% methylcellulose aqueous solution Source: Study Project 19-TX-0020

### Table 11 Toxicokinetics in 4-Week Repeated Dose Toxicity Study of Dapagliflozin and Project 19 in Rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Analyte** | **No.** | **Sex** | **Daily Dose**  **(mg/kg)** | **Cmax (ng/mL)** | | **AUC24 (ng•h/mL)** | | **Study No.** |
| **Day 1** | **Day 28** | **Day 1** | **Day 28** |
| **Dapagliflozin** | 3 | M | 0.1 | 63.9 | 38.3 | 596 | 285 | Project 19-TX-  0020 |
| 3 | M | 1 | 659 | 374 | 6450 | 2820 |
| 3 | M | 10 | 3850 | 3780 | 38400 | 24700 |
| 3 | M | 100 | 37600 | 32000 | 487000 | 395000 |
| **Project 19** | 3 | M | 1 | 185 | 253 | 1500 | 1220 |
| 3 | M | 10 | 1710 | 1590 | 11500 | 9710 |
| 3 | M | 100 | 12700 | 13500 | 135000 | 115000 |

Source: Study Project 19-TX-0020

### Effects of Combination Administration of Project 19 with Metformin in Rats

Prior to conducting a 13-week combination toxicity study of Project 19 with metformin, a preliminary 2-week toxicity study of metformin (dose level: 0, 100, 300, 1000 and

2000 mg/kg/day) was performed to obtain a toxicity profile and examine toxicokinetics in rats [[Table 12](#_bookmark83)]. In this study, a dose level of 2000 mg/kg/day was lethal. The dose level of 100 mg/kg/day was selected for the 13-week combination toxicity study with Project 19 based on toxicokinetic data which are equivalent to those at the maximum recommended human daily dose level (AUCinf or AUC72h at approximately 24 mcg·h/mL) [Cullen et al., 2004; Sambol et al., 1996].

In the 13-week combination toxicity study in rats, Project 19 was orally administered at dose levels of 0.1, 1, 10 and 100 mg/kg/day concomitantly with 100 mg/kg/day (as free form) of metformin hydrochloride (combination dosing groups) for 13 weeks to 10 male and

10 female rats per group to evaluate the effects of combination dosing (Study Project 19-TX- 0038). A 100 mg/kg/day Project 19 alone group, a 100 mg/kg/day metformin alone group, and a control group were also included. Systemic exposure to Project 19 and metformin were determined.

Dose dependent elevation of UGE was noted at 0.1 mg/kg/day of Project 19 or more in combination dosing groups, and reduction in plasma glucose was noted at 10 mg/kg/day or more, as pharmacological effects. The extent of these changes at 100 mg/kg/day of Project 19 in combination with 100 mg/kg/day of metformin was similar to that at

100 mg/kg/day of Project 19 alone.

In combination groups with metformin, the following changes were noted in a dose dependent manner at 1 mg/kg of Project 19 or more, and the extent of the changes was broadly similar at 100 mg/kg/day of Project 19 with metformin to those at 100 mg/kg/day of Project 19 alone. They included increased food and water consumption, increased urine volume, urinary creatinine excretion, creatinine clearance, and urinary electrolyte excretion, decreased urinary osmotic pressure, increased urinary ketone bodies, decreased plasma chloride and calcium, and decreased zymogen granules in the pancreas; increased urinary excretion of NAG and beta-2-MIC, increased BUN, increased kidney weight, and dilatation

of the renal tubules and hypertrophy of the collecting tubular epithelium in the kidney; regeneration of the mucosal epithelium, submucosal inflammation, and erosion in the stomach; increased AST and ALT; and decreased erythrocytes and a shortening of the blood coagulation time.

In toxicokinetics [[Table 13](#_bookmark84)], no interaction between Project 19 and metformin was noted.

From these results, no obvious difference was noted in the toxicological profile of Project 19 between dosing alone and dosing concomitantly with metformin. The NOAEL for Project 19 in this combination dosing study was determined to be 0.1mg/kg/day. This level was the same as the NOAEL established in the 13-week toxicity study of Project 19 and no synergistic effects of concomitant dosing were evident.

### Table 12 Toxicokinetics in Preliminary 2-Week Repeated Dose Combination Toxicity Study of Metformin in Rats

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Sex** | **Daily Dose**  **(mg/kg** | **Cmax (ng/mL)** | | **AUC24 (ng•h/mL)** | | **Study No.** |
| **Day 1** | **Day 14** | **Day 1** | **Day 14** |
| 3  3 | M  F | 100 | 5520 | 6690 | 27000 | 32800 | Project 19-TX-0037 |
| 5590 | 6660 | 25000 | 25900 |
| 3  3 | M  F | 300 | 10900 | 16300 | 81000 | 125000 |
| 14500 | 14000 | 85600 | 84900 |
| 3  3 | M  F | 1000 | 20500 | 24300 | 206000 | 274000 |
| 19300 | 24700 | 171000 | 232000 |
| 3  3 | M  F | 2000 | 34000 | NA | 365000 | NA |
| 40400 | NA | 323000 | NA |

NA: Not applicable because of animal death Source: Study Project 19-TX-0037

### Table 13 Toxicokinetics in 13-Week Repeated Dose Combination Toxicity Study of Project 19 with Metformin in Rats

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Analyte** | **No.** | **Sex** | **Daily Dose (mg/kg)** | | **Cmax (ng/mL)** | | **AUC24**  **(ng•h/mL)** | | **Study No.** |
| **Project 19** | **Metformin** | **Day 1** | **Day 91** | **Day 1** | **Day 91** |
| **Project 19** | 3  3 | M  F | 0 | 100 | NC | NC | NC | NC | Project 19- TX- 0038 |
| NC | NC | NC | NC |
| 3  3 | M  F | 100 | 0 | 19600 | 23800 | 235000 | 162000 |
| 30900 | 40000 | 419000 | 377000 |
| 3  3 | M  F | 0.1 | 100 | 22.2 | 22.7 | 178 | 141 |
| 34.7 | 56.6 | 303 | 343 |
| 3  3 | M  F | 1 | 100 | 215 | 235 | 1750 | 1530 |
| 332 | 712 | 2930 | 3120 |
| 3  3 | M  F | 10 | 100 | 2440 | 2600 | 23700 | 18400 |
| 3440 | 5450 | 33000 | 29700 |
| 3  3 | M  F | 100 | 100 | 17300 | 21300 | 218000 | 167000 |
| 30600 | 44600 | 464000 | 504000 |
| **Metformin** | 3  3 | M  F | 0 | 100 | 4240 | 5680 | 28500 | 31900 |
| 5170 | 6990 | 24700 | 25800 |
| 3  3 | M  F | 100 | 0 | NC | NC | NC | NC |
| NC | NC | NC | NC |
| 3  3 | M  F | 0.1 | 100 | 5100 | 6800 | 22400 | 25200 |
| 7210 | 5750 | 25200 | 26500 |
| 3  3 | M  F | 1 | 100 | 4850 | 6420 | 25300 | 28300 |
| 5340 | 6340 | 26500 | 29000 |
| 3  3 | M  F | 10 | 100 | 4780 | 6540 | 27500 | 30200 |
| 5490 | 7180 | 26700 | 28500 |
| 3  3 | M  F | 100 | 100 | 5100 | 5840 | 33900 | 34500 |
| 5380 | 5010 | 34100 | 32200 |

NC: Not calculated

Source: Study Project 19-TX-0038

### Hemolysis Test in Human Erythrocytes

The hemolytic action of Project 19 injection (0.05 mg/mL as Project 19) was examined in vitro using human erythrocytes (Study Project 19-TX-0041). It was concluded that Project 19 injection had no hemolytic action on human peripheral blood.

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

*Primary Pharmacology*

Project 19, a potent and highly selective inhibitor of human SGLT2, exerted good, long-term glycemic control without affecting body weight gain. Coadministration of

Project 19 with glibenclamide or metformin showed an additive antihyperglycemic effect

when compared with monotherapy alone. Project 19 interfered with glucose absorption in the small intestine (SGLT1-related) only at the highest dose tested (30 mg/kg). These results suggest that Project 19 may be an effective glycemic control agent both as monotherapy and as combination therapy with SUDs or metformin.

*Safety pharmacology*

In vitro and in vivo safety pharmacology study results raised no concerns with regard to the cardiovascular, respiratory and central nervous systems.

*Toxicology*

Nonclinical toxicity studies showed that the major target organs for Project 19 in the rat are the kidney, liver, gastrointestinal tract and adrenal medulla, while no major target organ was identified in monkeys.

A statistically significant decrease in plasma glucose levels was observed in rats at

10 mg/kg/day or more. In cynomolgus monkeys, 1 female at 10 mg/kg/day and 1 female at 300 mg/kg/day showed decreased plasma glucose levels only at Week 52. No symptoms indicative of hypoglycemia were recorded throughout the animal toxicity study.

Pharmacology-related UGE was observed in both rats and monkeys. In rats, increases in food intake, urine volume, water intake, electrolyte excretion and kidney weight and a decrease in body weight gain, glycogen deposition in renal tubules and enlargement of vacuoles in brown adipose tissue were evident. These were considered to be related to the pharmacological action of Project 19, namely osmotic diuresis, loss of calories or their compensatory changes which are caused by glycosuria.

With regard to the kidney, increased BUN, dilatation of renal tubules and hypertrophy of the renal tubular epithelium were noted. These changes are similar to those observed in rats under diuretic or fasting conditions [Ogino et al., 1994; Horiuchi et al., 1992; Kaissling and Stanton, 1988]; thus, they are also considered to be related to the osmotic diuresis caused by increased UGE.

Increases in urinary NAG and beta-2-MIC excretion were detected in rats and monkeys. Since urinary NAG and beta-2-MIC are known biomarkers for proximal tubular damage, exploratory studies were conducted using three different SGLT2 inhibitors, Project 19, YM543 and dapagliflozin. All 3 compounds showed potential to increase urinary NAG excretion in rats. Histopathology examination did not show any changes in the renal tissues including the proximal tubules. These data suggested the possibility that the elevation of urinary NAG excretion was not associated with proximal tubular damage but with the pharmacological activity, though the exact mechanism of NAG elevation induced by Project 19 is still unknown.

Unlike rats, kidney-related findings were not obvious in monkeys. This might be partly due to the lack of diuretic effects in monkeys which have lower urinary glucose concentrations than rats.

Increases in AST and ALT were noted in rats at 10 mg/kg/day or more. Histopathology examination did not detect any degenerative or necrotic changes in the liver tissue. It was reported that liver enzymes were increased in fasting rats [Horiuchi et al., 1992]; thus, increased liver enzymes observed in rats could be related to loss of calories due to increased UGE. In the monkey studies, increases in AST and ALT were not obvious.

Regarding the gastrointestinal tract, rat studies demonstrated mucosal damage in the stomach and duodenum. These changes were reversible. In monkey studies, no such mucosal damage in the gastrointestinal tract was induced.

The NOAEL in rats after 26 weeks of dosing was 0.1 mg/kg/day, which was the same as that after 13 weeks of dosing. In male monkeys, the NOAEL was 10 mg/kg/day regardless of the dosing period while in female monkeys, the NOAEL decreased to 1 mg/kg/day after 52 weeks of treatment compared to 10 mg/kg/day after 13 weeks of treatment. However, the findings including increases in urinary NAG and beta-2-MIC and plasma triglycerides observed at the next high dose level in the 52-week study were only mild and occurred in just 1 female monkey.

Regarding the adrenal medulla, a 104-week rat carcinogenicity study demonstrated that an increased incidence of adrenal medullary hyperplasia and pheochromocytoma was observed indicating the tumorigenic potential of Project 19 in rats. However, it is considered that the pheochromocytoma in rats is most probably induced by an increased intake of calcium and is unlikely to be relevant to humans (External Assessment Report Project 19-TX-0039 and Project 19-TX- 0040). There were no treatment-related increases in the incidence of any tumor in either sex in the 104-week carcinogenicity study in mice.

A 4-week repeated dose toxicity study of SGLT2 inhibitors in rats was performed with dapagliflozin as a reference compound. The study result showed that Project 19 had a comparable safety profile to dapagliflozin.

In order to investigate the effects of combination dosing of Project 19 and metformin, a

13-week combination toxicity study was conducted in rats. This study demonstrated that the nature and extent of the findings observed in the combination groups were comparable to those in the Project 19 alone group. Toxicokinetic parameters of Project 19 and metformin were unaffected by combination dosing.

Safety pharmacology studies raised no concerns regarding the cardiovascular, respiratory and central nervous systems.

Project 19 had no potential to cause genotoxicity, teratogenicity or skin-sensitization. Project 19 had potential to cause mild eye irritation.

Project 19 injection had no venous or perivascular irritancy to rabbits or no hemolytic action on human peripheral blood.

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