### Safety Pharmacology

The safety pharmacology studies included investigations into the influence of PROJECT Z on the rat central nervous system, the dog respiratory and cardiovascular systems, the hERG channel (IKr channel), and the isolated guinea-pig papillary muscle. A summary of the results is shown [in Table 4 -](#_bookmark45) 5. The doses of PROJECT Z are expressed as the amount of the parent drug, PROJECT Z, after correction for percent purity.

### Table 4 - 5 Summary of the Results of the Safety Pharmacology Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Study system | Species, Strain, | Method | End point(s) | Major findings | Study |
|  | Sex, No./group, |  | measured |  | No./GLP |
|  | Dosage, and |  |  |  | compliance |
|  | method of |  |  |  |  |
|  | administration |  |  |  |  |
| Central | Rat, SD, | Irwin’s | General activity, | No effect up to | Project Z-PT- |
| nervous | male, | method | behavior | 2000 mg/kg | 0001 |
| system | n=6/group, |  |  |  | /GLP |
| *in vivo* | 0, 100, 1000, |  |  |  |  |
|  | 2000 mg/kg, |  |  |  |  |
|  | Oral |  |  |  |  |
| Cardiovascular | hERG- | Whole cell | Inhibition of hERG | No effect up to | Project Z-PT- |
| system | transfected | patch clamp | current | 10 µmol/L | 0002 |
| *in vitro* | HEK293 cells, |  |  |  | /GLP |
|  | n=5/group, |  |  |  |  |
|  | 0, 0.1, 1, |  |  |  |  |
|  | 10 µmol/L |  |  |  |  |
| Cardiovascular | Isolated guinea- | Isolated | Action potential | No effect up to | Project Z-PT- |
| system | pig papillary | papillary | duration (APD), | 10 µmol/L | 0003 |
| *in vitro* | muscle | muscle, | resting membrane |  | /GLP |
|  | samples, | microelectrode | potential (RP), |  |  |
|  | Hartley, male, | method | action potential |  |  |
|  | n=5/group, 0, |  | amplitude (APA), |  |  |
|  | 0.1, 1, |  | maximum upstroke |  |  |
|  | 10 µmol/L |  | velocity (dV/dt max) |  |  |
| Respiratory | Dog, beagle, | Under | Respiration rate, blood gasa, blood pressure, heart rate, ECGb | No effect up to | Project Z-PT- |
| and | male, | unanesthetized | 2000 mg/kg | 0004 |
| cardiovascular | n=4/group, 0, | condition, |  | /GLP |
| systems | 10, 100, 1000, | telemetry |  |  |
| *in vivo* | 2000 mg/kg, |  |  |  |
|  | Oral |  |  |  |

a: Artery pH, arterial O2 pressure, arterial carbon dioxide pressure, and hemoglobin oxygen saturation. b: PR interval, QRS duration, QT interval, and QTc (Fridericia formula QT/(RR)1/3).

### Central Nervous System

PROJECT Z was given once orally to 6 male SD rats per group at doses of 100, 1000, and 2000 mg/kg. The animals were observed for any effect of PROJECT Z on the central nervous

system using Irwin’s method. PROJECT Z had no effect on general activity and behavior at any dose tested.

* + - 1. **Cardiovascular System (*In Vitro*)**

The effect of PROJECT Z on the hERG channel (IKr channel) current was examined in hERG- expressing HEK293 cells using a whole cell patch clamp technique. PROJECT Z was applied to the cells at concentrations of 0.1, 1, and 10 μmol/L. No inhibition of the hERG current was observed at concentrations up to and including 10 μmol/L.

The effect of PROJECT Z on isolated guinea-pig papillary muscle was examined using a microelectrode method. PROJECT Z was applied to the muscle at concentrations of 0.1, 1, and 10 μmol/L. No prolongation of the action potential duration was observed at concentrations up to and including 10 μmol/L.

### Respiratory and Cardiovascular Systems (*In Vivo*)

PROJECT Z was given once orally to 4 male beagle dogs at doses of 10, 100, 1000, and

2000 mg/kg. The animals were examined for any effect of PROJECT Z on the respiratory and cardiovascular systems using telemetry under unanesthetized condition. Using a Latin square design with a washout period, the animals were tested repeatedly and measured for respiration rate, blood gas, blood pressure, heart rate, and ECG up to 24 hours after administration. PROJECT Z had no effect on respiration rate, blood gas, blood pressure, heart rate, and ECG at any dose tested.

## Toxicology

Single- and repeated-dose toxicity studies in rats and dogs, genotoxicity studies in bacteria and mammalian cells, and reproductive and developmental toxicity studies in rats and rabbits were conducted to evaluate the safety of PROJECT Z. Single- and repeated-dose toxicity studies, genotoxicity studies, and reproductive and developmental toxicity studies were conducted in accordance with Ministry of Health, Labour and Welfare guideline and ICH guidelines. The route of exposure was oral, the intended clinical route. The doses of PROJECT Z are expressed as the amount of the parent drug, PROJECT Z, after correction for percent purity.

A list of toxicity studies is summarized in [Table 4 - 12.](#_bookmark79)

### Table 4 - 12 List of Toxicity Studies

|  |  |  |  |
| --- | --- | --- | --- |
| Type of study | Study | GLP  compliance | Study No. |
| Single-dose | Single-dose toxicity study in rats | Yes | Project Z-TX-0005 |
| Single-dose toxicity study in dogs | Yes | Project Z-TX-0006 |
| Repeated-dose | 1-week repeated-dose toxicity study in rats (dose range finding) | No | Project Z-TX-0001  Project Z-TX-0002 |
| 4-week repeated-dose toxicity study in rats | Yes | Project Z-TX-0006 |
| 1-week repeated-dose toxicity study in dogs (dose range finding) | No | Project Z-TX-0004 |
| 4-week repeated-dose toxicity study in dogs | Yes | Project Z-TX-0008 |
| Genotoxicity | Reverse mutation test | Yes | Project Z-TX-0009 |
| Chromosomal aberration test | Yes | Project Z-TX-0010 |
| Reproductive and developmental toxicity | Embryo-fetal development toxicity study in rats (dose range finding) | No | Project Z-TX-0011 |
| Embryo-fetal development toxicity study in rats | Yes | Project Z-TX-0012 |
| Embryo-fetal development toxicity study in rabbits  (dose range finding) | No | Project Z-TX-0013 |
| Embryo-fetal development toxicity study in rabbits | Yes | Project Z-TX-0014 |

### Single-dose Toxicity

Single-dose oral toxicity studies were conducted in rats and beagle dogs.

### Single-dose Toxicity Study in Rats

[Table 4 - 13](#_bookmark83) shows the study results in rats.

No remarkable findings were noted up to 2000 mg/kg in both male and female rats.

### Table 4 - 13 Summary of the Results of a Single-dose Toxicity Study in Rats

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Species, Strain,**  **Dosing route** | **No.,**  **Sex/ Group** | **Doses (mg/kg)** | **Death** | **Major Findings (mg/kg)** | **Study No./GLP**  **Compliance** |
| **Single- dose$** | Rat  SD | 5M  5F | 1000  2000 | No death | No remarkable findings | Project Z-TX-  0005/ |
|  | Oral |  |  |  |  | GLP |

$: A 14-day post-dose observation period was set.

### Single-dose Toxicity Study in Dogs

[Table 4 - 14](#_bookmark85) shows the study results in beagle dogs. [Table 4 - 15](#_bookmark86) shows the plasma drug concentrations measured.

No death was observed up to the highest dose of 2000 mg/kg in either males or females. As a major finding, loose stools were noted at 2000 mg/kg on Day 1. There were no abnormalities in the histopathological examinations.

### Table 4 - 14 Summary of the Results of a Single-dose Toxicity Study in Dogs

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of study** | **Species, Strain, Dosing**  **route** | **No.,**  **Sex/ Group** | **Doses (mg/kg)** | **Death** | **Major findings (mg/kg)** | **Study No./GLP**  **compliance** |
| **Single- dose$** | Beagle dog | 1 M  1 F | 1000  2000 | No death | 2000: loose stools (Day 1) | Project Z-TX-  0006/ |
|  | Oral |  |  |  |  | GLP |

$: A 14-day post-dose observation period was set.

### Table 4 - 15 Plasma Drug Concentrations in a Single-dose Toxicity Study in Dogs

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species, Strain, Dosing route, Vehicle, Duration**  **of dosing** | **No.** | **Sex** | **Doses (mg/kg)** | **Cmax (ng/mL)** | **AUC24h**  **(ng·h/mL)** | **Study No./GLP**  **compliance** |
| **Beagle dog, Oral, 0.5% methylcellulose solution, Single dose** | 1  1 | M F | 1000 | 2882.5  8932.1 | 23704.6  64238.0 | Project Z-TX-  0006/ GLP |
| 1  1 | M  F | 2000 | 3848.4  7420.4 | 24810.9  48035.7 |

### Repeated-dose Toxicity

Four-week repeated-dose oral toxicity studies with a 4-week recovery period were conducted in rats and dogs. A 1-week repeated-dose study was also conducted as a dose finding study (non-GLP dose finding study).

### Four-week Repeated-dose Toxicity Study in Rats

[Table 4 - 16](#_bookmark90) shows the study results. [Table 4 - 17](#_bookmark92) shows the plasma drug concentrations measured.

An increased liver weight was observed in males and an increased ALT level in females at doses of 300 mg/kg or higher. These changes were noted in both males and females at 2000 mg/kg, as well as an increase in the AST levels in females. In addition, increases in food consumption and cecum weight (including contents) were observed in both males and females at 2000 mg/kg. The increased ALT and AST levels were less than twice the upper limit of normal at doses of 300 mg/kg and 1000 mg/kg, while ALT levels more than twice the upper limit of normal were seen in some females at 2000 mg/kg. However, a histopathological examination identified no abnormality in any of these tissues. Except for the increase in food consumption in males, these changes were resolved after a 4-week discontinuation of the drug. The NOAEL was estimated to be 100 mg/kg/day.

### Table 4 - 16 Summary of the Results of Repeated-dose Toxicity Studies in Rats

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of study** | **Species, Strain,**  **Dosing route** | **No./Sex/ Group** | **Doses (mg/kg)** | **Death** | **Major findings (mg/kg)** | **Study No.**  **/GLP**  **compliance** |
| **1-week** | Rat | 4 M | 0  10a)  30  100 a)  300  1000 | No death | 1000: ↑ AST (F), ALT (F), | Project Z-TX- |
| **repeated-** | SD | 4 F |  | liver weight (M) | 0001, |
| **dose** | Oral |  |  |  | Project Z-TX- |
| **(dose** |  |  |  |  | 0002 |
| **range** |  |  |  |  | /Non-GLP |
| **finding)** |  |  |  |  |  |
| **4-week** | Rat | 10 M | 0 | No death | 300: ↑ ALT (F), liver weight (M)  2000: ↑ FC (M, F), ALT (M),  AST (F), liver weight (F), cecum weight (M, F) | Project Z-TX- |
| **repeated-** | SD | 10 F | 100 |  | 0007 |
| **dose** | Oral | (5 M for | 300 |  | /GLP |
| **(with 4-** |  | recovery, 5 | 1000 |  |  |
| **week** |  | F/0 or 2000 | 2000 |  |  |
| **recovery)** |  | mg/kg) |  |  |  |

a): Males only. The underlined dose is the estimated NOAEL.

### Table 4 - 17 Plasma Drug Concentrations in Repeated-dose Toxicity Studies in Rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species, Strain, Dosing route, Vehicle, Duration of**  **dosing** | **No.** | **Sex** | **Dose (mg/kg)** | **Cmax (ng/mL)** | | **AUC24h (ng·h/mL)** | | **Study No.**  **/GLP**  **compliance** |
| First dose | Last dose | First dose | Last dose |
| **Rat, SD, Oral, 0.5%**  **methylcellulose solution,**  **1 week** | 2  0 | M  F | 10 | 20  — | 53  — | 130  — | 191  — | Project Z-TX-  0001  Project Z-TX-  0002  /Non-GLP |
| 2  2 | M  F | 30 | 298  61 | 102  129 | 1206  188 | 499  421 |
| 2  2 | M  F | 100 | 345  82 | 483  — | 3953  249 | 1194  — |
| 2  2 | M  F | 300 | 385  113 | 254  438 | 2777  705 | 1585  2541 |
| 2  2 | M  F | 1000 | 496  536 | 483  481 | 4744  5695 | 5303  3478 |
| **Rat, SD, Oral, 0.5%**  **methylcellulose solution,**  **4 weeks** | 3  3 | M  F | 100 | 138.2  152.6 | 207.8  159.8 | 1272.4  1012.9 | 1498.7  1203.3 | Project Z-TX-  0007  /GLP |
| 3  3 | M  F | 300 | 265.2  260.5 | 280.5  359.0 | 2289.4  1735.8 | 2853.7  2710.4 |
| 3  3 | M  F | 1000 | 320.5  421.4 | 543.5  534.6 | 3127.3  2756.0 | 5539.1  3077.3 |
| 3  3 | M  F | 2000 | 487.6  538.5 | 861.0  794.2 | 4199.6  3782.6 | 6846.8  5892.9 |

The underlined dose is the estimated NOAEL.

### Four-week Repeated-dose Toxicity Study in Dogs

[Table 4 - 18](#_bookmark95) shows the study results. [Table 4 - 19](#_bookmark96) shows the plasma drug concentrations measured.

Abnormalities in feces such as loose and mucoid stools were observed at 1000 mg/kg throughout the treatment period, and weight loss was noted after the 4-week administration. However, no abnormality was found in any of the tissues in the histopathological examination. The abnormalities in the feces disappeared on the third day after discontinuation of the drug. Weight loss was also improved after discontinuation of the drug. The NOAEL was estimated to be 300 mg/kg/day.

### Table 4 - 18 Summary of the Results of Repeated-dose Toxicity Studies in Dogs

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of study** | **Species, strain,**  **dosing route** | **No./Sex/ Group** | **Doses (mg/ kg)** | **Death** | **Major findings (mg/kg)** | **Study No.**  **/GLP**  **compliance** |
| **1-week** | Beagle dog | 1 M | 0 | No | 1000: vomiting, loose stool | Project Z-TX- |
| **repeated-** | Oral | 1 F | 10 | death | (M), ↓ plasma triglyceride, | 0004 |
| **dose** |  |  | 100 |  | inorganic phosphate, ↑ | /Non-GLP |
| **(dose** |  |  | 1000 |  | chloride (M, F) |  |
| **range** |  |  |  |  |  |  |
| **finding)** |  |  |  |  |  |  |
| **4-week** | Beagle dog | 3 M | 0 | No | 1000: loose stool, mucoid | Project Z-TX- |
| **repeated-** | Oral | 3 F | 30 | death | stool (M, F), weight loss (M) | 0008 |
| **dose** |  | (3 M for | 100 |  |  | /GLP |
| **(with 4-** |  | recovery, 3 | 300 |  |  |  |
| **week** |  | F/1000 mg/kg) | 1000 |  |  |  |
| **recovery)** |  |  |  |  |  |  |

The underlined dose is the estimated NOAEL.

### Table 4 - 19 Plasma Drug Concentrations in Repeated-dose Toxicity Studies in Dogs

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species, Strain, Dosing route, Vehicle, Duration of**  **dosing** | **No.** | **Sex** | **Doses (mg/kg)** | **Cmax (ng/mL)** | | **AUC24h (ng·h/mL)** | | **Study No.**  **/GLP**  **compliance** |
| First dose | Last dose | First dose | Last dose |
| **Beagle dog, Oral, 0.5% methylcellulose solution, 1 week** | 1  1 | M  F | 10 | 851  1660 | 801  972 | 8988  10619 | 8072  9112 | Project Z-TX-0004  /Non-GLP |
| 1  1 | M  F | 100 | 2740  913 | 2130  2120 | 8486  10646 | 13860  16740 |
| 1  1 | M  F | 300 | 3370  6810 | 8060  5040 | 15662  47949 | 50212  33231 |
| **Beagle dog, Oral, 0.5% methylcellulose solution, 4 weeks** | 3  3 | M  F | 30 | 1211.2  1280.1 | 1579.7  1031.8 | 8876.3  11888.3 | 9903.5  10445.9 | Project Z-TX-0008  /GLP |
| 3  3 | M  F | 100 | 1561.7  1812.4 | 2184.2  1840.3 | 11706.8  8229.8 | 12439.2  14500.7 |
| 3  3 | M  F | 300 | 2733.0  1820.9 | 2920.8  1991.7 | 17702.7  13315.9 | 18625.8  12044.2 |
| 6  6 | M  F | 1000 | 3463.6  3950.4 | 2956.1  2545.5 | 19086.2  19998.3 | 13315.5  11563.0 |

The underlined dose is the estimated NOAEL.

### Genotoxicity

[Table 4 - 20](#_bookmark99) shows the study results.

A bacterial reverse mutation test did not identify an increase in the number of revertant colonies regardless of the presence or absence of metabolic activation (S9). PROJECT Z was not found to induce gene mutation. An *in vitro* mammalian chromosome aberration test did not identify an increase in the frequency of chromosomally-aberrant cells either with or without metabolic activation. PROJECT Z was not found to induce chromosomal aberrations in cells.

On the basis of these results, PROJECT Z was considered not to be genotoxic.

### Table 4 - 20 Summary of the Results of Genotoxicity Studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, particulars** | **No./Sex/Group** | **Doses** | **Result** | **Study No./GLP**  **compliance** |
| **Reverse** | *S. typhimurium* (TA98, | — | S9-: 0, 6.86– | Negative | Project Z-TX- |
| **mutation** | TA100, TA1535, |  | 5000 |  | 0009 |
|  | TA1537), *E.coli* |  | µg/plate |  | /GLP |
|  | (WP2*uvrA*), Preincubation |  | S9+: 0, |  |  |
|  | method (with or without a |  | 6.86–5000 |  |  |
|  | metabolic activation |  | µg/plate |  |  |
|  | system [rat liver S9]) |  |  |  |  |
| **Chromosomal** | CHL/IU cells, Treatment | — | 6h (S9-): 0, | Negative | Project Z-TX- |
| **aberration** | for 6 h (with or without a |  | 625, 1250, |  | 0010 |
|  | metabolic activation |  | 2500, |  | /GLP |
|  | system [rat liver S9]) and |  | 5000 µg/mL |  |  |
|  | for 24 h (without rat liver |  | 6h (S9+): 0, |  |  |
|  | S9) |  | 625, 1250, |  |  |
|  |  |  | 2500, |  |  |
|  |  |  | 5000 µg/mL |  |  |
|  |  |  | 24h (S9-): 0, |  |  |
|  |  |  | 625, 1250, |  |  |
|  |  |  | 2500, |  |  |
|  |  |  | 5000 µg/mL |  |  |

### Carcinogenicity

Not performed.

### Reproductive and Developmental Toxicity

The influence of PROJECT Z on embryo-fetal development was investigated in rats and rabbits. Preliminary studies of the influence of PROJECT Z on embryo-fetal development in rats and rabbits were also performed (non-GLP studies).

### Embryo-fetal Development Toxicity Studies in Rats

[Table 4 - 21](#_bookmark105) shows the study results. The only effect on dams was an increase in food consumption observed at 1000 mg/kg or higher. There was no effect on embryo-fetal

development and no teratogenic potential was noted. Therefore, the NOAELs for dams and embryo-fetal development were estimated to be 100 mg/kg/day and 2000 mg/kg, respectively.

### Embryo-fetal Development Toxicity Studies in Rabbits

[Table 4 - 21](#_bookmark105) shows the study results. There was no drug-related effect on the dams or on embryo-fetal development up to 1000 mg/kg, and no teratogenic potential was noted.

Therefore, the NOAELs for both dams and embryo-fetal development were estimated to be 1000 mg/kg/day.

### Table 4 - 21 Summary of the Results of Reproductive and Developmental Toxicity

**Studies**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study, Duration of dosing** | **Species, strain,**  **dosing route** | **No./Sex/Group** | **Doses (mg/kg)** | **Death** | **Major findings (mg/kg)** | **Study No.**  **/GLP**  **compliance** |
| **Embryo- fetal**  **development** | Rat SD  Oral | 5-6  F | 0  100  1000  2000 | No death | Effect on F0  100: ↑ FC  Effect on F1  ≤2000: Not remarkable | Project Z-TX-  0011  Non-GLP |
| **(dose range** |  |  |  |
| **finding),** |  |  |  |
| **Days 7 to 17** |  |  |  |
| **of gestation** |  |  |  |
| **Embryo- fetal development,**  **Days 7 to 17** | Rat SD  Oral | 19-20  F | 0  100  1000  **2000** | No death | Effect on F0  1000: ↑ FC  Effect on F1  ≤2000: Not remarkable | Project Z-TX-  0012  GLP |
| **of gestation** |  |  |  |  |  |
| **Embryo- fetal development** | Rabbit NZW  Oral | 3  F | 100  300  1000 | No death | ≤1000: Not remarkable | Project Z-TX-  0013  Non-GLP |
| **(dose range** |  |  |  |
| **finding),** |  |  |  |
| **Non-** |  |  |  |
| **pregnant,** |  |  |  |
| **5 days** |  |  |  |
| **Embryo- fetal**  **development, Days 6 to 18** | Rabbit NZW  Oral | 18-20  F | 0  100  300  **1000** | No death | Effect on F0  ≤1000: Not remarkable Effect on F1  ≤1000: Not remarkable | Project Z-TX-  0014  GLP |
| **of gestation** |  |  |  |  |  |

The underlined doses are the estimated NOAELs for general toxicity.

The bolded doses are the estimated NOAELs for embryo-fetal development.

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

PROJECT Z is an alpha-amylase inhibitor. Alpha-amylase, one of digestive enzymes found in pancreatic fluid and saliva, converts starch (polysaccharide) into maltose (disaccharide) by hydrolysis of the glucoside bonds. PROJECT Z is expected to inhibit the activity of alpha- amylase and block the hydrolysis of polysaccharide into disaccharide and thus to block glucose absorption and suppress postprandial hyperglycemia. Alpha-glucosidase inhibitors, on the other hand, competitively inhibit the disaccharide degrading enzyme (alpha- glucosidase), which is found in small intestine epithelial cells, blocking hydrolysis of maltose and sucrose (disaccharides) into glucose (monosaccharide). Alpha-glucosidase inhibitors may induce osmotic diarrhea associated with the accumulation of disaccharides and fluid within the gastrointestinal tract. Alpha-amylase inhibitors cause the accumulation of starch in the gastrointestinal tract and therefore the accumulation of disaccharides is much lower compared to alpha-glucosidase inhibitors, and thus is not expected to cause osmotic diarrhea.

PROJECT Z showed a potent inhibitory effect on pancreatic amylases from animals (mice, rats, dogs, monkeys, and humans) and on human saliva amylase, while it had almost no inhibitory effect on small intestinal disaccharidases (sucrase, maltase, isomaltase, lactase, and trehalase) in animals.

A single dose of PROJECT Z suppressed the elevation of blood glucose in KK-Ay mice after carbohydrate solution loading, and repeated-doses of PROJECT Z (3 times/day for 2 weeks) improved HbA1c levels. With these results, PROJECT Z is expected to improve HbA1c through its inhibitory effect on the elevation of postprandial blood glucose levels in the clinical setting. The minimum effective dose of PROJECT Z required for suppressing the elevation of blood glucose in KK-Ay mice after carbohydrate solution loading is 1 mg/kg.

The diarrhea-inducing activity of PROJECT Z was investigated in KK-Ay mice with voglibose, an alpha-glucosidase inhibitor, as a control drug. While voglibose demonstrated a significant diarrhea-inducing activity at 0.3 mg/kg, PROJECT Z did not cause diarrhea even at 10 mg/kg.

These results suggest that PROJECT Z has potential as a drug with less diarrhea-inducing activity than voglibose in patients with diabetes mellitus.

Single oral administration of PROJECT Z at 30 mg/kg to rats and at 1, 3, and 10 mg/kg to dogs resulted in low absolute bioavailabilities (2.3% in rats and 6.0% to 13.2% in dogs).

After single oral administration of radiolabeled PROJECT Z (14C-PROJECT Z) at 30 mg/kg to pigmented rats, the radioactivity concentrations in the gastrointestinal tract and its contents were substantially higher than those in other tissues. Moderate radioactivity concentrations were found in some organs (excluding the gastrointestinal tract) such as the kidneys,

pancreas, Harderian gland, submandibular gland, and liver. Radioactivity persisted in the organs for a relatively long time and was detected 672 hours after administration in the Harderian gland, submandibular gland, liver, spleen, pancreas, pigmented skin, as well as white skin. The *in vitro* plasma protein binding of PROJECT Z in mice, rats, rabbits, dogs, monkeys, and humans was approximately 70% to 80% and no major differences among species were observed.

Most of the fecal radioactivity after single oral administration of 14C-PROJECT Z at 30 mg/kg to rats was due to metabolites. Most of the administered radioactivity was considered to be degraded or metabolized in the gastrointestinal tract thereafter excreted in the feces.

PROJECT Z did not show noticeable inhibition of CYP1A2, 2C8, 2C9, 2C19, 2D6 or 3A4 activity in human liver microsomes up to 300 μmol/L.

The excretion rates of radioactivity in urine, feces, and expired air up to 168 hours after

single oral administration of 14C-PROJECT Z at 30 mg/kg to rats were 10.8 ± 3.1%, 84.1 ± 2.8%, and 1.1 ± 0.4%, respectively. It is considered that, while part of the radioactivity is absorbed in the body, most of it is unabsorbed and excreted in the feces.

In the non-clinical safety assessment, safety pharmacology studies, single-dose toxicity studies, 4-week repeated-dose toxicity studies, genotoxicity studies, and embryo-fetal development toxicity studies were completed. Pivotal toxicity studies and safety pharmacology studies were conducted in compliance with GLP regulations.

PROJECT Z mainly targets the gastrointestinal tract and the liver.

Gastrointestinal toxicities with the primary symptoms being abnormalities in feces (e.g. loose and mucoid stools) were observed in dogs at high doses of 1000 mg/kg or more. However, no abnormality was detected in the gastrointestinal tissues. The changes were reversible and rapidly resolved after discontinuation of the drug. Therefore, clinical studies are considered to be feasible with close monitoring of the subjects for such symptoms.

The effects on the liver, which resulted in an increase in blood ALT and AST levels as well as an increase in liver weight, were observed in rats at doses of 300 mg/kg or higher.

However, no abnormality was detected in the liver tissues. These changes were reversible and resolved after discontinuation of the drug. Therefore, clinical studies are considered to be feasible with careful monitoring including conducting liver function tests in the subjects.

There were no toxicological findings in the safety pharmacology studies, genotoxicity studies, and embryo-fetal development studies.

The intended starting dose of PROJECT Z in a clinical study (Project Z-CL-0101) is 0.3 mg/man, which represents a dose more than 10 times lower than the HED (4.8 mg) at the minimal effective dose in the non-clinical pharmacology study in mice (1 mg/kg) and 3000 times lower than the HED (960 mg) estimated using the NOAEL in the 4-week repeated-dose toxicity study in rats showing high sensitivity to PROJECT Z (100 mg/kg/day). Thus, the starting dose in the proposed clinical study is considered to be low enough to ensure safety.

### Table 4 - 22 Comparison of the Minimum Effective Dose and Human Equivalent Dose (HED) Based on the NOAEL of PROJECT Z in Animals with the Starting Dose in Humans

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Sex (M/F)** | **Dose (mg/kg/day)** | **HEDa**  **(mg/60 kg)** | **Cmax (ng/mL)** | | **AUC24h (ng·h/mL)** | | **Study No.** |
| First  dose | Week 4 | First dose | Week 4 |
| **Mouse, NA/ST Z**  **KK-Ay** | M | 1  (Minimum effective dose) | 4.8 | — | | — | | Project Z-PH-0016  Project Z-PH-0018 |
| **SD**  **Rat** | M  F | 100  (NOAEL) | 960 | 138.2  152.6 | 207.8  159.8 | 1272.4  1012.9 | 1498.7  1203.3 | Project Z-TX-0007 |
| **Beagle dog** | M  F | 300  (NOAEL) | 9720 | 2733.0  1820.9 | 2920.8  1991.7 | 17702.7  13315.9 | 18625.8  12044.2 | Project Z-TX-0008 |
| **Human** | M | 0.3  (Starting dose) | 0.3 | — | | — | | Project Z-CL-0001 |

a: The human equivalent dose (HED) levels were calculated by using the body surface area factors (mouse, 3; rat, 6; dog, 20; human, 37) and a human body weight of 60 kg.