### Safety Pharmacology

PROJECT 3 had no effect on central nervous system (general activity or behavior) in male rats at doses up to 300 mg/kg po (Project 3-PT-0004).

In male dogs PROJECT 3 resulted in vomiting and diarrhea at 30 mg/kg po or more. PROJECT 3 has no effect on general activity, behavior, body temperature, cardiovascular system (blood pressure, heart rates, and electrocardiography including QT interval), respiratory system (respiration rates and blood gas parameters), or blood-electrolyte concentrations (sodium, ionized calcium, potassium, and chloride) at doses up to 300 mg/kg po (Project 3-PT-0003).

PROJECT 3 had no effect on action potential duration using cardiac papillary muscles isolated from guinea pigs at concentrations up to 1x10-5M (Project 3-PT-0002).

PROJECT 3 had no effect on hERG current in hERG channel-transfected HEK293 cells at concentrations up to 1x10-6M (Project 3-PT-0001). At 1x 10-5M, the current was inhibited by 18.7%. The magnitude of inhibition was small and the concentration of 1x 10-5M is far beyond the free fraction concentration achieved in a clinical setting. Furthermore, no alterations were detected in the action potential duration assay and the safety pharmacology study in dogs. Therefore, the potency of hERG current inhibition recorded in HEK293 cells was considered not to be of clinical relevance.

### Table 5 Safety pharmacology studies of PROJECT 3

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Type of study** | **Test System** | **Species, Strain, Sex, Dosing**  **particulars** | **End Point(s) Measured** | **Major Findings** | **Study No.** |
| **hERG current** | whole cell patch clamp | hERG channel transfected HEK293 cells n=5  0, 1x10-7M, 1x10-  6M, 1x10-5M | inhibition of hERG current | 18.7% inhibition at 1x10-5M | Project 3- PT- 0001 |
| **Action potential duration** | glass electrode technique | papillary muscles isolated from Hartley guinea pigs  n=5  0, 1x10-7M, 1x10-  6M, 1x10-5M | resting membrane potential, action potential amplitude, dV/dtmax, action potential duration (APD30, APD90,  APD30–90) | No changes | Project 3- PT- 0002 |
| **Central nervous system** | modified Irwin’s method | rat, SD, male n = 6/dose group  0, 30, 100,  300 mg/kg | general activity, behavior | No changes | Project 3- PT- 0004 |
| **Cardiovascular and respiratory system** | telemetry | dog, beagle, male n = 4/dose group 0, 3, 30,  300 mg/kg | general activity, behavior, body temperature, blood pressure, heart rate, ECG parameters, respiration rates, blood-gas parameters,  blood-electrolyte concentrations | 30: vomiting, diarrhea | Project 3- PT- 0003 |

### Pharmacodynamic Drug Interactions

There are no nonclinical pharmacodynamics drug interactions studies of PROJECT 3.

### Other Pharmacology Studies

There are no nonclinical other pharmacology studies of PROJECT 3.

## Toxicology

### Single-dose Toxicity

Acute toxicity of PROJECT 3 was assessed in rats and dogs. PROJECT 3 was given once orally to rats and dogs at doses of 1000 and 2000 mg/kg and the animals were observed for 14 days.

Since no animals were dead at the highest dose of 2000mg/kg, the lethal dose was more than 2000 mg/kg both in rats and dogs.

In rats, suppressed body weight gain, decreased locomotor activity, and abnormal gait were observed at 1000 mg/kg or more (Project 3-TX-0001). In dogs, watery or soft stool, and prolonged activated partial thromboplastin time (APTT) were observed at 1000 mg/kg or more (Project 3-TX-0002). These findings occurred transiently on Day 1 (the day of dosing) or Day 2.

### Table 6 Single-dose toxicity studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain,**  **Dosing particulars** | **No./ Sex/ Dose** | **Doses (mg/kg)** | **Death** | **Major findings** | **Study No.** |
| **Single- dose** | Rat, SD,  Oral gavage, observation  for 14 days | 5M, 5F | 0  1000  2000 | No death | Suppressed body weight gain, decreased locomotor activity, abnormal gait | Project 3- TX- 0001 |
| **Single- dose** | Dog, Beagle, Oral gavage, observation  for 14 days | 1M, 1F | 1000  2000 | No death | Soft stool, watery stool, APTT prolongation | Project 3- TX- 0002 |

### Repeat-dose Toxicity

Repeated-dose toxicity studies of PROJECT 3 were conducted in rats and dogs.

### Four-week repeat dose oral toxicity study in rats

One-week dose range finding studies in rats demonstrated that the dose group of 600 mg/kg severely deteriorates condition of the rats. Therefore, a 4-week oral toxicity study in rats was conducted at dose levels of 3, 10, 30, and 300 mg/kg/day (Project 3-TX-0003). The following toxicologically significant findings were observed only at the highest dose of 300 mg/kg:

* + - * + Hematology and bone marrow examination showed mild decreases in erythrocyte counts, Hb, Hct, and platelet counts and an increase in orthochromatic erythroblast ratio.
        + Blood chemistry showed increases in AST, ALT, LDH, ALP, total cholesterol, and creatinine, and decreases in glucose, triglyceride, total protein, albumin, globulin, and potassium. The extent of the changes of these biochemical parameters was within 2- fold of the concurrent control values.
        + In urinalysis, decreased osmolality were observed.
        + Weight of the heart and liver was increased. The heart weight was increased by approximately 10%. Weight of salivary glands and ovaries was decreased. Histopathological examination showed centrilobular hepatocyte hypertrophy, mild or minimal single cell necrosis in the exocrine pancreas, minimal transitional cell hypertrophy of the urinary bladder. Electronmicroscopy for hypertrophied centrilobular hepatocytes showed proliferation of smooth endoplasmic reticulum.
        + In addition, microvesiculation of the white adipocytes and macrovesiculation of the brown adipocytes were noted. The pathological diagnosis, microvesiculation and macrovesiculation, means reduced and increased size of a lipid vacuole in each adipocyte. These changes in size are not related to histological damage. Therefore, these histological changes in adipocytes were not considered to be toxicologically significant.

At 30 mg/kg or more, unidentified crystals in urinary sediment were observed in urinalysis in male rats only. The urinary crystals alone were considered to be of no toxicological significance.

Collectively the NOAEL is judged as 30 mg/kg/day.

After the 4-week recovery period, all findings other than the change of salivary gland weight were recovered. Since the change of salivary gland weight was accompanied with neither histological changes nor clinical signs, it was considered to be of no toxicological significance.

### Thirteen-week repeat dose oral toxicity study in rats

A 13-week oral toxicity study in rats was conducted at dose levels of 3, 10, 30, 100, and 300 mg/kg/day (Project 3-TX-0017). Similar as in the 4-week study, toxicologically significant findings were observed only at the highest dose of 300 mg/kg.

* + - * + Hematology showed mild decreases in erythrocyte counts, Hb, Hct, and platelet counts. Unlike the 4-week study, no changes were noted in bone marrow examination.
        + Blood chemistry showed increases in AST, ALT, CPK, ALP and creatinine, and decreases in total protein, albumin, globulin, A/G ratio and potassium. The magnitude of these changes was within 2-fold of the concurrent control values. Unlike the 4- week study, there were no changes in blood glucose levels.
        + Urinalysis showed increases in water intake and urine volume accompanying with low urine osmolality.
        + Weight of the heart and liver was increased. The heart weight was increased by approximately 30%. Unlike the 4-week study, the weight of the salivary glands and ovary was not affected.
        + Histopathology examination showed mild hypertrophy of centrilobular hepatocytes, minimal single cell necrosis in the exocrine pancreas, minimal hypertrophy of transitional cell epithelium in the urinary bladder and renal pelvis. These findings were similar to those observed in the 4-week study. In addition, minimal focal necrosis of hepatocytes and minimal hypertrophy of adrenal cortex (zona glomerulosa) were recorded at 300 mg/kg/day.
        + Histological alterations in the adipose tissues, which are not toxicologically significant, were noted at a lower dose. These are similar to the results in the 4-week study.
        + Similar to the 4-week rat study, male rat-specific unidentified crystal formation was detected in urinalysis. Cloudy yellow color urine was noted in males at

300 mg/kg/day, which was considered to be caused by the unidentified crystals. In addition, calcium oxalate crystal was detected in females at 300 mg/kg/day. The crystals alone were considered to be of no toxicological significance.

Statistically significant decreases for blood triglyceride values were noted for males at

30 mg/kg/day or more. The potential beneficial effects on TG reduction at 30 mg/kg/day was not considered to be of toxicological relevance and is not impacting on the NOAEL Collectively, the NOAEL is judged as 100 mg/kg/day.

Reversibility of all these toxicity findings was confirmed following to the 4-week follow-up period.

### Table 7 Repeat-dose toxicity studies in Rats

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **particulars** | **No./Sex/ Dose** | **Doses (mg/kg)** | **Deaths** | **Major findings** | **Study No.** |
| **4-week repeat- dose**  **with**  **4-week recovery** | Rat, SD,  Oral gavage | 10M,  10F  5M, 5F  for recovery | 0  3  10  **30**  300 | No death | Hematology  300: ↓ erythrocyte, Hb, Hct, platelet,  ↑ orthochromatic erythroblast ratio in bone marrow  Liver  300: ↑ AST, ALT, LDH, ALP, total  chol., liver weight, hypertrophy and sER proliferation of centrilobular hepatocytes  ↓ TP, albumin  Kidney function and Electrolytes 300: ↑ creatinine  ↓ urine osmolality, blood K Urinary tract system  300: minimal mucosal hypertrophy of urinary bladder (no correlation with crystal formation was confirmed based on individual data)  Exocrine pancreas  300: mild or minimal single cell necrosis of exocrine acinus Other changes  30: microvesiculation of white adipocytes male rat-specific urinary unidentified crystals  300: ↑ heart weight  ↓ glucose, TG, globulin, salivary gland weight, ovary weight macrovesiculation of brown adipocytes | Project 3- TX- 0003 |
|  |  |  |  |  | Findings after recovery period 300: ↓ salivary gland weight |  |
| **13-week repeat- dose**  **with**  **4-week recovery** | Rat, SD,  Oral gavage | 10M,  10F  5M, 5F  for recovery | 0  3  10  30  **100**  300 | No death | Hematology  300: ↓ erythrocyte, Hb, Hct, platelet (no changes in the marrow)  Liver  300: ↑ AST, ALT, ALP, liver weight  ↓ TP, albumin  centrilobular hypertrophy, minimal focal necrosis  Kidney function and Electrolytes 300: ↑ creatinine, water intake, urine volume  ↓ urine osmolality, blood K Urinary tract system  300: minimal mucosal hypertrophy (bladder and renal pelvis) (no correlation with crystal formation  was confirmed based on individual | Project 3- TX- 0017 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **particulars** | **No./Sex/ Dose** | **Doses (mg/kg)** | **Deaths** | **Major findings** | **Study No.** |
|  |  |  |  |  | data)  Exocrine pancreas  300: minimal single cell necrosis of exocrine acinus  Other changes  30: male rat-specific urinary unidentified crystals, ↓ TG (male)  100: microvesiculation of white adipocytes, macrovesiculation of brown adipocytes  300: calcium oxalate urinary crystals (female only), cloudy yellow urine due to unidentified crystals (male only)  ↑ heart weight, CPK  ↓ globulin  minimal hypertrophy in adrenal zona glomerulosa  Findings after recovery period No (All findings recovered) |  |

The NOAELs are bold and underlined

### Four-week repeat dose oral toxicity study in dogs

A 4-week repeated-dose study in dogs was performed at dose levels of 1, 3, 30, and 300 mg/kg/day (Project 3-TX-0004).

Toxicologically significant findings were observed at 30 or 300 mg/kg, or both. Vomiting, soft, mucous, or watery stool, and salivation were observed dose-dependently. Soft stool was also recorded on the first day of the recovery period. Decreased body weight or body weight gain and decreased food consumption were recorded in several dogs.

Hematology and bone marrow examination showed decreases in erythrocyte counts, Hb, Hct, reticulocyte counts, and myeloid/erythroid cell ratio and an increase in polychromatic erythroblast ratio. The findings in the bone marrow were considered to be compensatory response to the low erythroid parameters.

Blood chemistry showed increases in urea nitrogen, and creatinine and decreases in calcium, total protein, albumin, and globulin. The extent of the changes of these biochemical parameters was within 2-fold of the concurrent control values.

Pathological examination showed increases in the liver and adrenal weights, extramedullary hematopoiesis in the spleen, decreased glycogen deposition in hepatocytes, and microvesiculation of the white adipocytes.

At 3 mg/kg, microvesiculation of the white adipocytes was observed in one female out of 6 dogs. Microvesiculation means reduced size of a lipid vacuole in each adipocyte. This change is not related to histological damages. Therefore, microvesiculation was not considered to be of toxicological significance and the dose of 3 mg/kg is judged as the NOAEL.

After the 4-week recovery period, all findings were recovered

### Thirteen-week repeat dose oral toxicity study in dogs

A 13-week repeated-dose study in dogs was performed at dose levels of 0.3, 1, 3 and 30 mg/kg/day (Project 3-TX-0018).

Toxicologically significant findings were noted only at 30 mg/kg. Soft, mucous, or watery stool and salivation were recorded as clinical signs.

* + - * + Hematology showed decreases in erythrocyte counts, Hb, Hct, and reticulocyte counts. Bone marrow examination showed a marginal increase in basophilic erythroblasts ratio. This bone marrow change was considered to be of compensatory response to the low erythroid parameters. This bone marrow change was also detected after a 4-week follow-up period.
        + Marginal elevation of neutrophil count was recorded in a male dog. Since pathology examination did not detect any inflammation in this dog, this neutrophil elevation was considered to be a chance finding.
        + Blood chemistry showed increases in ALT and urea nitrogen and decreases in total cholesterol, albumin and calcium. The extent of the changes of these biochemical parameters was within 2-fold of the concurrent control values.
        + Increased organ weight changes were noted in the heart and liver. The heart weight was increased by approximately 30%. Minimal atrophy of the sebaceous glands was observed microscopically. Histological alteration in the adipose tissues, which are not toxicologically significant, was recorded. This was similar to the results in the 4-week study.

Collectively the dose of 3 mg/kg is judged as the NOAEL.

### Table 8 Repeat-dose toxicity studies in Dogs

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **particulars** | **No./Sex/ Dose** | **Doses (mg/kg)** | **Deaths** | **Major findings** | **Study No.** |
| **4-week repeat- dose**  **with**  **4-week recovery** | Dog, Beagle, Oral gavage | 3M, 3F  3M, 3F  for recovery | 0  1  **3**  30  300 | No death | Gastro-intestine  30: vomiting, salivation, soft stool, mucous stool, watery stool Hematology  30: ↓ erythrocyte, Hb, Hct, hematopoiesis in spleen  300: ↓ reticulocyte, M/E ratio, responsive marrow change (↑ polychromatic erythroblasts ratio) Liver  30: ↓ TP  300: ↓ albumin, ↑ liver weight, decreased glycogen deposition in hepatocytes  Kidney function and Electrolytes 300: ↑ creatinine, UN, ↓ Ca Others  3: microvesiculation of white adipocytes  30: ↓ BW gain, BW, FC  300: ↓ globulin, ↑ adrenal weight | Project 3- TX-0004 |
|  |  |  |  |  | Findings after recovery period No (All findings recovered) |  |
| **13-week repeat- dose**  **with**  **4-week recovery** | Dog, Beagle, Oral gavage | 3M, 3F  3M, 3F  for recovery | 0  0.3  1  **3**  30 | No death | Gastro-intestine  30: salivation, soft stool, mucous stool, watery stool  Hematology  30: ↓ erythrocyte, Hb, Hct, reticulocyte, neutrophil, responsive marrow change (↑ basophilic erythroblasts ratio)  Liver  30: ↑ ALT, liver weight  ↓ total chol. albumin  Kidney function and Electrolytes 30: ↑ UN, ↓ Ca  Others  30: ↑ heart weight, microvesiculation of white adipocytes, minimal atrophy of sebaceous gland | Project 3- TX-0018 |
|  |  |  |  |  | Findings after recovery period  responsive marrow change (↑ basophilic erythroblasts ratio) |  |

The NOAELs are bold and underlined

### Genotoxicity

A reverse mutation test showed negative results (Project 3-TX-0009).

An increase in frequency (7.5%) of chromosomal aberrant cells in a short-term (6 hours) exposure without metabolic activation mixture was noted only at the highest concentration of 180 µg/mL in the chromosomal aberration test using Chinese hamster lung cells (CHL/IU cells) (Project 3-TX-0012). At this dose, the number of viable cells was 39.1% of those at the control.

The micronucleus test using mice bone marrow cells (250, 500, or 1000 mg/kg was orally given in mice for 2 days) (Project 3-TX-0010) and an unscheduled DNA synthesis (UDS) test using rat hepatocytes (500, 1000, or 2000 mg/kg was given in rats once orally) showed negative results (Project 3-TX-0011).

The plasma exposure levels (AUC0–24) at the highest dose in the micronucleus test and the UDS test were 3563 and 2824 µg.h/mL and these large exposures were sufficient to assess the genotoxic potential.

Since no genotoxicity was shown in the reverse mutation test, micronucleus test and UDS test and the clastogenic effect in in vitro chromosomal aberration test was only observed at the highest 180 µg/mL where the cytotoxicity was greater than 50%, it was considered that PROJECT 3 has no genotoxicity under in vivo conditions and the clastogenic potential is not relevant to human risk.

### Table 9 Genotoxicity studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **particulars** | **No./Sex/ Dose** | **Doses** | **Major findings** | **Study No.** |
| **Reverse mutation test** | S. typhimurium (TA100, TA1535, TA98, TA1537)  E.coli (WP2*uvrA*) | - | 5–5000 µg/plate | No increase in revertant colonies | Project 3-TX-  0009 |
| **Chromosomal aberration test** | CHL/IU cells Treatment for 6h with or without metabolic activation system  (S9) and for 18h without S9 | - | 6h -S9: 25–  180 µg/mL  6h, +S9: 25–  160 µg/mL  24h, -S9: 6.25–  120 µg/mL | 7.5% increase in aberrant cells in 6h treatment without S9 at 180 µg/mL 1) | Project 3-TX-  0012 |
| **Micronucleus test** | Mouse, ICR, oral gavage for 2 days | 5M, 5F | 0  250  500  1000 | No increase in micronucleated polychromatic erythrocytes in  femoral bone marrow | Project 3-TX-  0010 |
| **Unscheduled DNA**  **synthesis test** | Rat, SD, oral gavage, single | 3M | 0  500  1000  2000 | No increase in net nuclear grain counts or incidence of cells in repair in isolated  hepatocytes | Project 3-TX-  0011 |

1): the viable cell count ratio at 180 μg/mL: 39.1%

### Carcinogenicity

No information available yet.

### Reproductive and Developmental Toxicity

### Embryo-fetal development

Assessments for embryo-fetal development were performed in rabbits and rats. The results showed PROJECT 3 has no teratogenicity.

Increased placental weights and skeletal variations in fetuses were recorded at the highest dose of the rat study (Project 3-TX-0007). The NOAEL for the dams and fetuses was 100 mg/kg. In the dose range finding study for rabbits, death, abortion, and reduced body weights were observed at 300 mg/kg. Therefore, 100 mg/kg was set as the highest dose of the pivotal rabbit study. No findings in dams or fetuses were seen in the pivotal rabbit study (Project 3-TX-0008).

For rats, suppressed body weight gain and decreased food consumption were observed in dams at the highest of 300 mg/kg. Increased placental weights and increased frequency of cervical rib and 14th rib were seen in the fetuses at 300 mg/kg. Although placental weights were affected, there were no changes in the number of post-implantation loss and live fetuses or fetal body weight. Increased frequency of cervical rib and 14th rib are not considered to be of teratogenicity but rather variations because these changes occur spontaneously and not adversely affect survival or development of fetuses. Other than the variations, no abnormalities were detected in external, visceral and skeletal examinations.

### Fertility and early embryonic development to implantation

Effects on fertility and early embryonic development to implantation were assessed using male rats and female rats.

No test article-related effects were noted in the indices of fertility in males and females including days until copulation, copulation index, fertility index or number of corpora lutea in any treatment groups. Moreover, no test article-related effects were noted in the indices of early embryonic development until implantation including number of implantations, pre- and post-implantation loss indices or number of live embryos in any treatment groups.

In conclusion, the NOAELs of PROJECT 3 was judged as 300 mg/kg/day for fertility of males and females and early embryonic development.

### Pre- and postnatal development

No information available yet.

### Table 10 Reproductive toxicity studies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Species, Strain, Dosing**  **particulars** | **No./Sex/ Dose** | **Doses (mg/kg)** | **Major findings** | **Study No.** |
| **ICH-3:**  **Embryo-fetal development** | Rat, SD, oral gavage dosing during days 7 to 17 of gestation | 20F | 0  30  **100**  300 | Dams  300: ↓ BW gain, FC Fetuses  300: ↑ placenta weight, skeletal variations  (cervical rib, 14th rib) | Project 3-TX-  0007 |
| Rabbit, New Zealand white  oral gavage dosing  during days 6 to 18 of gestation | 19F, 20F,  or 21F | 0  10  30  **100** | Dams  no findings Fetuses  no findings | Project 3-TX-  0008 |
| **ICH-1:**  **Fertility and early embryonic development to implantation** | Male Rat, SD, oral gavage, 2 weeks prior to mating and throughout mating  to the day before necropsy | 20M | 0  30  100  **300** | No effects | Project 3-TX-  0016 |
| Female Rat, SD,  oral gavage, 2 weeks prior to mating through day 7 of gestation | 20F | 0  30  100  **300** | No effects | Project 3-TX-  0016 |

The NOAELs are bold and underlined

### Local Tolerance

Not applicable

### Other Toxicity Studies

Not applicable

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

PROJECT 3 is a GPR40 agonist with little species difference among human, mouse and rat in agonistic activities for GPR40. GPR40 is highly expressed in pancreas β-cells and its natural endogenous ligands are medium- and long-chain free fatty acids. Similar to those effects by free fatty acids, acute activation of β-cell GPR40 receptors by PROJECT 3 enhances GSIS). In *ob/ob* mice, a type 2 diabetic animal model, PROJECT 3 reduced both HbA1c levels and plasma insulin levels after 4-week once daily repeated dosings, suggesting that PROJECT 3 might improve insulin sensitivity after prolonged treatment.

Rats and dogs were chosen in the repeated-dose toxicity studies. The selection of animal species was justified as each human metabolite was detected at least in rats, dogs or rabbits in in vitro studies using cryopreserved hepatocytes.

Major non-clinical adverse events of PROJECT 3 were GI tract-related clinical signs (vomiting, and watery stool), low erythroid parameters (red blood cell count, hemoglobin, hematocrit), effects on the liver (mild increases in ALT, AST and ALP, minimal focal necrosis of hepatocytes in rats), effects on the renal function (mild increases in creatinine and urea nitrogen, increased urine volume, low urine osmolality, and marginal changes in blood potassium and calcium levels), minimal hypertrophy in the urothelial mucosa, heart weight increase, and exocrine pancreas (mild single cell necrosis in the exocrine acinar cells). All adverse findings were mild in nature and reversible.

These adverse findings were observed at 300 mg/kg/day in rats (AUC0–24: 539–

2019 µg.h/mL) and at 30 mg/kg/day in dogs (AUC0–24: 201–535 µg.h/mL). These AUC exposure levels at LOAEL considerably exceeded the human exposure level at 1200 mg qd (AUCinf: 124–171 µg.h/mL) (Project 3-CL-0001).

The 13-week rat study showed focal hepatocyte necrosis at 300 mg/kg/day. The severity of this necrosis was minimal and was not detected in the 4-week rat study. The AST, ALT and ALP values remained below two-fold of placebo values. The AUC at NOAEL of

100 mg/kg/day was 192–436 µg.h/mL. This NOAEL exposure level was beyond the highest exposure in the Project 3-CL-0001 study (124–171 µg.h/mL at 1200 mg). Taken collectively, the toxicological significance of the minimal hepatocyte necrosis in rats was limited.

The 4-week and 13-week rat studies showed male rat-specific urinary unidentified crystal formation. Since microscopic examination for the urinary sediment revealed the shape of the unidentified crystals was not similar to the ordinary endogenous crystals, the unidentified crystals are considered to be made of exogenous constituents. Mechanisms of the male-rat specific unidentified crystal formation are under investigation.

In addition, calcium oxalate urine crystals were detected in female rats of the 13-week study. On the other hand, none of dog studies (4-week and 13-week) showed urine crystal formation.

Urinary crystals alone are not considered as adverse findings unless the crystals led to mucosal injury for the urinary tract system. At the high dose of 300 mg/kg/day, minimal hypertrophy occurred in the urinary tract system. In the planned 26-week rat study, the association of histological alteration in the urinary tract with urinary crystal formation will be carefully investigated.

The risk of causing hypoglycemia under fasting conditions is expected to be low based on the mode of action of PROJECT 3. Non-clinical toxicity studies did not show any risk of hypoglycemia. Statistically significant reduction of blood glucose levels were recorded for female rats at the highest tested dose of 300 mg/kg in the 4-week study. However, the magnitude of the change was minimal (105 mg/dL *vs* concurrent control value of 114 mg/dL) and the other repeated studies (13-week rat, 4-week dog and 13-week dog studies) did not show any changes in the blood glucose levels so that this statistical difference is not expected to be in a clinical setting.

Pancreatic endocrine β-cell is one of the pharmacologically target tissues. The repeated-dose toxicity studies microscopically examined pancreatic endocrine islet cells including β-cells. No histological changes were detected up to 13 weeks administration.

Preliminary in vitro results showed that PROJECT 3 has a weak potential of activating PPAR receptors. It is considered that some of the toxicity findings might be related to the PPAR agonistic effects.

In vitro microsomal studies showed PROJECT 3 inhibitory activities on CYP2C8-mediated metabolisms. The potential of clinical drug-drug interaction with CYP2C8 substrates cannot be excluded.