### 4.1.3 Safety Pharmacology

Safety pharmacology studies were conducted to assess the potential effects of PROJECT C on hERG current in vitro, cardiovascular and respiratory systems in dogs and CNS in rats. All studies were performed in accordance with GLP. Noteworthy findings from safety pharmacology studies are provided in [End-of-Text Table 1.2].

### 4.1.3.1 In Vitro Effects on hERG Current

The effects of PROJECT C on the hERG current were studied in hERG-transfected HEK293 cells by the whole-cell patch-clamp technique (Study Project C-PT-0003). When PROJECT C was applied, the compensated suppression rates at the concentrations of 0.201, 0.661 and

2.16 µmol/L were 2.4%, 10.1% and 18.6%, respectively; statistically significant differences between PROJECT C and the vehicle control were noted at 0.661 and 2.16 µmol/L. The IC50 value of PROJECT C was not determined due to limited solubility.

### In Vivo Effects on CNS in Rats

PROJECT C (dose levels: 0, 10, 300 or 2000 mg/kg) was orally administered as a single dose to Sprague-Dawley rats (6 males per dose) and the CNS effects (general physical condition and behavior) were evaluated (Study Project C-PT-0001).

At 10 and 300 mg/kg, PROJECT C had no discernible effect on the CNS; while at 2000 mg/kg, a decreased body temperature was observed.

### In Vivo Effects on Cardiovascular and Respiratory Systems in Dogs

The effects of a single oral administration of PROJECT C (dose levels: 0, 1, 3, 10 or 100 mg/kg) on the cardiovascular and respiratory systems were evaluated in conscious beagle dogs

(4 males per dose) (Study Project C-PT-0002).

At 10 mg/kg, vomiting was noted. At 100 mg/kg, vomiting, salivation and a decrease in blood ionized potassium levels were observed. No discernible effects on cardiovascular or respiratory systems were noted at doses up to 100 mg/kg.

## Toxicology

A total of 12 GLP toxicity studies (including in vitro studies) and 2 non-GLP studies were conducted in mice, rats, dogs and rabbits. Additionally, 1 GLP study was conducted to elucidate the mechanism of toxicity observed. A tabulated overview of the nonclinical toxicology studies is provided in [End-of-Text Table 3.1].

### Single-dose Toxicity

No GLP single-dose toxicity studies have been conducted with PROJECT C as of the preparation of this IB.

### Repeated-dose Toxicity

Pivotal 4- and 13-week repeated-dose toxicity studies were conducted in rats and dogs.

### Pivotal 4-week Repeated-dose Toxicity Study in Rats with 4-week Recovery Period

PROJECT C was suspended in 0.5 w/v% MC solution and orally administered once daily for

1. weeks at dose levels of 0 (vehicle control), 10, 300 and 2000 mg/kg per day to 10 male and 10 female Crl:CD(SD) rats (7 weeks old at initiation of dosing) per group. Five males and
2. females were added to the control and 2000 mg/kg dose groups to assess the reversibility of toxicity observed during the dosing period in a subsequent 4-week recovery period

(Study Project C-TX-0001).

No toxicological changes were noted in either sex at 10 mg/kg or in females at 300 mg/kg.

At doses ≥ 300 mg/kg in males, body weight loss or low body weight gain on day 3 and food consumption on day 1 to 3 were noted. Hypertrophy of the follicular cells in the thyroids was observed in male rats.

At 2000 mg/kg, a decrease in spontaneous activity and traces of reddish rhinorrhea were observed in males early in the dosing period. Low body weight and food consumption, low erythrocyte count, hemoglobin concentration and hematocrit value, and high thyroid weight with hypertrophy of the follicular cells was observed in both females.

With dose increase, tmax delayed and Cmax and AUC24 increased less than dose proportionally. For Cmax and tmax, there was no repeated-dose dose effect or sex difference during the dosing period. For AUC24, no sex difference was observed; and values after repeated dose (days 14 and 28) tended to be low compared with the values on day 1.

The NOAEL was 10 mg/kg for males and 300 mg/kg for females. The changes observed during the dosing period fully recovered by the end of the 4-week recovery period.

### Pivotal 13-week Repeated-dose Toxicity Study in Rats with 4-week Recovery Period

PROJECT C was suspended in 0.5 w/v% MC solution and orally administered once daily for 13 weeks at dose levels of 0 (vehicle control), 10, 100 and 2000 mg/kg per day to 10 male and 10 female Crl:CD(SD) rats (7 weeks old at initiation of dosing) per group. Five males and 5 females were added to the control and 2000 mg/kg dose groups to assess the reversibility of toxicity observed during the dosing period in a subsequent 4-week recovery period (Project C-TX-0007).

No test article-related toxic changes were noted at PROJECT C doses of 10 or 100 mg/kg.

At 2000 mg/kg, 1 female rat was found dead on day 73 of dosing and this animal showed marked body weight loss on days 3, 21, 56 and 70 of dosing with low food consumption, degeneration and necrosis of the centrilobular hepatocytes and atrophy of the thymus (with macroscopic small size), submandibular lymph nodes, mesenteric lymph nodes and Peyer’s patches in addition to changes similar to those in the surviving animals. For the surviving animals at the same dose level, low body weight and food consumption in both sexes, a decrease in spontaneous activity in males and trace of reddish rhinorrhea in females were observed at the early stage of the dosing period. Low erythrocyte count, hemoglobin concentration and hematocrit value and high reticulocyte ratio were noted in both sexes.

In the toxicokinetics, group mean tmax delayed with the dose increase on day 1 of dosing, but there was no dose difference in tmax on day 49 or 91 of dosing. Group mean Cmax increased less than dose proportionally, and mean AUC24 increased dose proportionally (10 to

100 mg/kg) or less than dose proportionally (100 to 2000 mg/kg). Cmax and AUC24 showed no repeated-dose effect or sex difference during the dosing period except for males at

2000 mg/kg. Cmax and AUC24 in males at 2000 mg/kg after repeated dose (days 49 and 91) tended to be low compared with those on day 1 of dosing.

The NOAEL was 100 mg/kg in both sexes. The changes observed during the dosing period recovered during the 4-week recovery period.

### Pivotal 4-week Repeated-dose Toxicity Study in Dogs with 4-week Recovery Period

PROJECT C was suspended in 0.5 w/v% MC solution and orally administered once daily for 4 weeks at dose levels of 0 (vehicle control), 1, 3, 10 and 100 mg/kg per day to 4 male and 4 female beagle dogs per group. Three males and 3 females were added to the 100 mg/kg

dose group to assess the reversibility of toxicity during a subsequent 4-week recovery period (Study Project C-TX-0002).

No test article-related changes were noted at 1 or 3 mg/kg in either sex or at 10 mg/kg in females.

At 10 mg/kg, vomiting was observed in 1 male mainly immediately after dosing or 1 to 2 h after dosing between week 1 and 3 of dosing; and salivation was observed in males immediately after dosing.

At 100 mg/kg, 1 male was sacrificed on day 14 of dosing. The animal showed a severe decrease in spontaneous activity, vomiting, somnolence and ataxic gait. Low food consumption was noted from day 4 of dosing, and body weight on day 14 of dosing was decreased by approximately 25% from that on day -1. These changes were almost identical to those in the scheduled sacrificed animals. No food consumption was noted on the day before sacrifice and a severe decrease in spontaneous activity on the morning of day 14. In histopathology, atrophy of the thymus and a decrease in pancreatic zymogen granules were observed.

At 100 mg/kg of the surviving animals, vomiting, salivation, somnolence, ataxic gait and a decrease in spontaneous activity were observed. Severe decreases in body weights and food consumption were observed. Low heart rate and prolongation of QT and QTcM in 1 male were noted on day 22. In urinalysis, high bilirubin and glucose and low sodium excretion, potassium and chloride excretion were noted. Hematological findings included low erythrocyte count, hemoglobin concentration and hematocrit value as well as high fibrinogen in males, while females showed high erythrocyte count, hemoglobin concentration, hematocrit value and platelet count (1 female) as well as decreased lymphocyte counts. In clinical chemistry, the increases ALT, globulin and sodium (1 female), the increases total protein, albumin, glucose and chloride in females, the increases total cholesterol in 1 male and females, the increases urea nitrogen in 1 male, and the decreases sodium and chloride in 1 male were noted. Small-sized thymus and low thymus weights was observed in 1 male and 1 female, and moderate atrophy of the thymus and a slight decrease in pancreatic zymogen granules in histopathology were observed in these animals. Low testes weights, marked atrophy of the seminiferous tubules in the testes and absence of the sperm in the epididymides in histopathology were observed in only 1 male.

Mean toxicokinetic parameters (tmax, Cmax and AUC24) generally increased with increasing dose level. After repeated dosing, mean toxicokinetic parameters were approximately the same at 1, 3 and 10 mg/kg except for mean AUC24 values in males at 10 mg/kg, which increased after repeated dosing. Mean toxicokinetic parameters at 100 mg/kg increased or tended to increase after repeated dosing except for mean tmax values in females, which were approximately the same after repeated dosing. There was no clear sex difference in any toxicokinetic parameter.

It was concluded that the NOAEL was 3 mg/kg for males and 10 mg/kg for females, and 100 mg/kg was considered to exceed the maximum tolerated dose (MTD). While the reversibility of low heart rate, QT prolongation and the effect on the testes could not be confirmed due to a low incidence, other findings observed during the dosing period were reversible at the end of the recovery period.

### Pivotal 13-week Repeated-dose Toxicity Study in Dogs with 4-week Recovery Period

PROJECT C was suspended in 0.5 w/v% MC solution and orally administered once daily for 13 weeks at dose levels of 0 (vehicle control), 3, 10 and 30 mg/kg per day to 4 male and

4 female beagle dogs per group. Three males and 3 females were added to the 30 mg/kg dose　group to assess the reversibility of toxicity during a subsequent 4-week recovery period (Study Project C-TX-0008).

No test article-related toxic changes were noted at 3 mg/kg.

At 10 mg/kg, vomiting was observed in all males and 1 female mainly 1 to 2 or 4 to 6 h after dosing at weeks 1 or 2 of dosing, and salivation was observed in 1 male immediately after dosing between week 9 and week 13 of dosing.

At 30 mg/kg, vomiting was observed in all males and females mainly before dosing, 1 to 2 h after dosing or 4 to 6 h after dosing between week 1 and week 7 of dosing, and salivation was observed in 5 males and 6 females mainly immediately after dosing or 1 to 2 h after dosing during the dosing period. Somnolence was observed in 2 males and 1 female 1 to 2 h after dosing on day 2, and ataxic gait was observed in 4 males and 4 females almost 1 to 2 h after dosing in week 1 of dosing. A decrease in spontaneous activity was observed in 1 male 1 to 2 h after dosing on day 1 of dosing. Low erythrocyte count was noted in males and 1 female, and low hemoglobin concentration and hematocrit value were noted in 1 female. High ALT, triglycerides and total cholesterol in 1 female each, high creatinine in females, and high urea nitrogen in 1 female were noted. Low absolute and relative pancreas weights were noted in females and high absolute and relative thyroid weights were noted in 1 female.

During the recovery period, no test article-related changes were noted in any examination.

In the toxicokinetics, the group mean Cmax and AUC24 values increased with increasing dose level. As an exception, mean Cmax and AUC24 values at 30 mg/kg in males on day 91 and mean AUC24 value at the same dose level in females on day 1 were almost the same as the value at 10 mg/kg. Group mean tmax values were between 1.0 and 8.8 h. After repeated dosing, there were no clear differences in any toxicokinetic parameter with the exception of mean tmax values in females at 10 mg/kg and in males at 30 mg/kg. Mean tmax values in females at 10 mg/kg and in males at 30 mg/kg on day 1 were higher than those values on days 49 and 91. There were no clear sex differences in Cmax and AUC24.

The NOAEL was 3 mg/kg in both sexes. No test article-related changes were noted in any examination during the recovery period; therefore, the changes observed during the dosing period were judged to be reversible.

### Genotoxicity

The genotoxic potential of PROJECT C was evaluated with 2 in vitro tests (reverse mutation test and chromosome aberration test) and 1 in vivo test (micronucleus in rats).

### In Vitro Reverse Mutation Test in Bacteria

A bacterial reverse mutation test was performed with and without metabolic activation at 4.69, 9.38, 18.8, 37.5, 75 and 150 µg/plate (Study Project C-TX-0003).

In comparison with the negative control, no 2-fold or greater increase in the number of revertant colonies was observed in any test strain with or without metabolic activation.　It was concluded that PROJECT C has no potential to induce gene mutation in bacteria under the conditions of this study.

### In Vitro Chromosome Aberration Test in Cultured Mammalian Cells

A chromosome aberration test was performed with cultured mammalian (Chinese hamster lung fibroblast [CHL/IU]) cells with and without metabolic activation at 39.1, 62.5 and 100 µg/mL in short-term treatment and 24.4, 39.1 and 62.5 µg/mL in continuous treatment for 24 h (Study Project C-TX-0004).

No significant increase in the number of cells with structural or numerical chromosomal aberrations was noted in any treatment group when compared with those in the negative control group.

It was concluded that PROJECT C has no potential to induce chromosomal aberrations in CHL/IU cells regardless of the presence or absence of metabolic activation or treatment length.

### In Vivo Micronucleus Test in Rats

PROJECT C was suspended in 0.5 w/v% MC solution and administered twice with a 24-h interval at a dosing volume of 10 mL/kg per day. As a negative control, the vehicle was administered in the same manner.

PROJECT C was orally administered to male Crl:CD(SD) rats twice with a 24-h interval at dose levels of 0 (0.5 w/v% MC solution), 500, 1000 and 2000 mg/kg (Study Project C-TX-0009).

Approximately 24 h after the second dosing, femoral bone marrow specimens were prepared, and the number of micronucleated immature erythrocytes (MNIE) per 2000 immature erythrocytes (IE) and the ratio of IE per 500 total erythrocytes were investigated.

A decrease in spontaneous activity (slight) was observed in 1 male in the 2000 mg/kg group, and a decrease in body weight was noted in all test article groups.

No statistically significant increase was noted in the number of MNIE in any group when compared with the negative control group. No statistically significant decrease was noted in the ratio of IE in any group when compared with the negative control group.

It was concluded that, under the conditions of this study, PROJECT C did not induce micronuclei in rat erythroblasts.

### Carcinogenicity

No carcinogenicity studies have been conducted with PROJECT C as of the preparation of this IB. For the purpose of finding a dose for a 104-week and 26-week planned carcinogenicity studies in rats and mice, respectively, a 13-week and 4-week dose-range finding toxicity studies were conducted.

### A 13-Week Oral Dose-range Finding Study for Carcinogenicity of PROJECT C in Rats

For the purpose of dose selection for a 104-week carcinogenicity study of PROJECT C in rats, a 13-week dose-range finding toxicity study was conducted (Project C-TX-0015). PROJECT C was administered orally by gavage to male and female RccHan:WIST rats (10/sex/group, 6 weeks of age at the start of treatment) for 13 weeks, at dose levels of 0 (0.5 w/v% MC solution), 100, 500 and 1500 mg/kg per day.

At 100 mg/kg, lower values for body weight and food consumption were noted in the early phase of the administration period in males.

At 500 mg/kg, lower values for body weight and food consumption were noted in the early phase of the administration period in both sexes.

At 1500 mg/kg, a decrease in spontaneous movement was observed in a few males and females at 4 h after dosing on day 1 and/or day 2 of administration. Lower values for body weight were noted in the early to middle phase of the administration period in both sexes, and lower values for food consumption were also noted in the early phase of the administration period.

As described above, mortality was not noted, repeated dosing at 1500 mg/kg caused some changes in rats such as transient suppression of body weight, low food consumption and abnormal clinical signs; however, a dose up to 1500 mg/kg was well tolerated.

### A 4-Week Oral Dose-range Finding Study for Carcinogenicity of PROJECT C in Nontransgenic Littermates of Jic:CB6F1-Tg rasH2@Jcl Mice

For the purpose of dose selection for a 26-week oral dose carcinogenicity study of PROJECT C in Tg rasH2 mice, a 4-week dose-range finding toxicity study was conducted in non-Tg littermates of rasH2 mice (Project C-TX-0017). PROJECT C was administered orally by gavage to male and female non-Tg rasH2 mice (10/sex/group, 8 weeks of age at the start of treatment) for 4 weeks, at dose levels of 0 (0.5 w/v% MC solution), 100, 500 and 1500 mg/kg per day.

At 100 mg/kg, lower values for food consumption were noted in the early phase of the administration period in both sexes.

At 500 mg/kg, a decrease in spontaneous movement was observed in a few males and females at 1 and/or 4 h after dosing on day1 and/or day 2 of administration. Lower values for body weight and food consumption were noted in the early phase of the administration period in both sexes. In the blood chemistry, an increase in potassium was noted in females.

At 1500 mg/kg, a decrease in spontaneous movement was observed in all males and females mainly 1 and/or 4 h after dosing on the first 3 days. Lower values for body weight and food consumption were noted in the early phase of the administration period in both sexes and on the contrary, thereafter higher values for food consumption were noted transiently in males. In the hematology, decreases in white blood cell and lymphocyte counts were noted in both sexes. In the blood chemistry, increases in potassium and blood urea nitrogen (BUN) were noted in females.

As described above, mortality was not noted, and repeated dosing at 1500 mg/kg caused several changes in mice such as transient suppression of body weight, decreased food consumption and abnormal clinical signs, decreased white blood cell and lymphocytes counts, and increases in potassium and BUN; however, a dose up to 1500 mg/kg was well tolerated.

### Reproductive and Developmental Toxicity

An embryo-fetal development study in rats and in rabbits (dose-range finding study) were conducted.

### Study for Effects of PROJECT C on Embryo-fetal Development in Rats

PROJECT C was administered orally from days 7 to 17 of gestation at dose levels of 0 (0.5 w/v% MC solution), 10, 300 or 2000 mg/kg per day to pregnant Crl:CD(SD) rats

(Study Project C-TX-0011). The dams were necropsied on day 20 of gestation. In dams, no test article-related changes were noted at 10 mg/kg. Decreases in body weight and food consumption were noted during the dosing period at 300 and 2000 mg/kg. No test

article-related changes were noted in the number of corpora lutea or number of implantations in any group. In fetuses, no test article-related changes were noted in the number of live fetuses, number of embryo-fetal deaths, postimplantation loss rate, sex ratio, fetal body weight, placental weight, or external or placental findings in any group, or in visceral or skeletal findings in the 2000 mg/kg group.

It was concluded that the NOAEL of PROJECT C was 10 mg/kg for dams and 2000 mg/kg for embryo-fetal development in rats.

### Study for Effects of PROJECT C on Embryo-fetal Development in Rabbits

PROJECT C was administered orally from day 6 to 18 of gestation at dose levels of 0 (0.5 w/v% MC solution), 10, 30 or 100 mg/kg per day to pregnant Kbl:NZW rabbits (Study Project C-TX- 0013). The dams were necropsied on day 28 of gestation.

In dams, decreases in fecal amount, food consumption and body weight were observed dose-dependently at 30 and 100 mg/kg. No abnormalities were noted in gross pathological findings at necropsy, and no treatment-related effects were noted in maintenance of pregnancy, in the numbers of corpora lutea, implantations, or the incidences of pre- implantation loss, or implantation at any dose. In fetuses, there were no test article-related effects on embryo-fetal development as seen in sex ratio and in the fetal mortality and

morphology. No suppression of fetal growth as expressed by fetal body weight and placental weight including the progress of ossification was observed in any group.

It was concluded that the NOAEL of PROJECT C was 10 mg/kg for effects on dams and 100 mg/kg for embryo-fetal development in rabbits.

### Local Tolerance

No local tolerance studies have been conducted with PROJECT C as of the preparation of this IB.

### Other Toxicity Studies

* + - 1. **In Vitro Phototoxicity**

The potential phototoxicity of PROJECT C was assessed in vitro in Balb/c 3T3 cells

(Study Project C-TX-0005). The cells were cultured with PROJECT C at concentrations of 4.74, 6.64, 9.30, 13.0, 18.2, 25.5, 35.7 and 50 µg/mL in the presence and absence of UV-A irradiation. PROJECT C was categorized as having no phototoxicity because the mean photo effect (actual value: 0.006) was less than 0.15.

### Serum Thyroid and Thyroid-stimulating Hormone Levels, Hepatic Enzyme Activities and Enzyme Gene Expression in Rats

Study Project C-TX-0010 was conducted to investigate the effects on the serum thyroid and thyroid-stimulating hormone levels, hepatic enzyme activity and hepatic enzyme gene expression in rats with thyroidal follicular cell hypertrophy induced by PROJECT C treatment for 1 week.

PROJECT C was orally administered to 10 male Crl:CD(SD) rats per group once daily at dose levels of 0 (0.5 w/v% MC solution), 300 and 2000 mg/kg per day for 1 week. The following observations and examinations were performed: clinical signs, body weight, food consumption, gross pathology, organ weight (thyroid glands and liver), histopathology (thyroid glands), serum thyroid hormone (thyroxine and triiodothyronine) and thyroid- stimulating hormone levels, hepatic enzyme activity (*p*-nitrophenol UDPGT) and gene expression (Ugt1a1, Ugt1a6, Ugt2b1 and Ugt2b2).

No test article-related changes were observed in clinical signs, gross pathology, organ weight, histopathology, serum thyroid hormone or thyroid-stimulating hormone levels or hepatic enzyme activity at 300 or 2000 mg/kg. Low body weight and food consumption were observed at 300 and 2000 mg/kg. Increased mRNA expression was observed in Ugt2b1 at 300 and 2000 mg/kg and Ugt1a1 and Ugt1a6 at 2000 mg/kg.

Under the conditions of this study, PROJECT C treatment did not induce thyroidal follicular cell hypertrophy, change in the serum hormone levels or change in the hepatic enzyme activity.

However, increased hepatic enzyme gene expression (Ugt2b1, Ugt1a1 and Ugt1a6) suggested that PROJECT C has a potential for hepatic UDPGT induction.

## Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

### Pharmacological Properties

PROJECT C is a novel compound with GABAB receptor PAM activity. In an intracellular Ca2+ mobilization assay using human and mouse GABAB receptor-expressing cells, PROJECT C enhanced the potency and efficacy of GABA in a concentration-related manner. PROJECT C at any concentration tested did not affect intracellular Ca2+ mobilization in the presence of the low concentrations of GABA (0.001 – 0.01 μmol/L), indicating PROJECT C had no direct agonist effects on GABAB receptors.

PROJECT C at a concentration of 10 µmol/L had no appreciable affinity (less than or close to 50% inhibition displacement of specific binding) to GABAB receptor orthosteric sites or 53 other receptors, ion channels and transporters. Additionally, the inhibitory effect of PROJECT C on 3 enzymes was less than 50%.

PROJECT C (1, 3 and 10 mg/kg po) decreased self-administrations of EtOH in male and female rats. Intragastric administration of PROJECT C (0.3, 1 and 3 mg/kg) to rhesus monkeys decreased self-administrations with morphine. PROJECT C at 3 and 10 mg/kg did not potentiate respiratory suppression induced by morphine in cynomolgus monkeys. In the rat CPP study, PROJECT C (1, 3 and 10 mg/kg po) suppressed the increase in cocaine-induced CPP score in a dose-related manner. Intragastric administration of PROJECT C at 0.1, 0.3 and 1 mg/kg in 1 out of 3 rhesus monkeys significantly decreased the mean number of self-administrations with cocaine.

Overall, the findings in rats and monkeys indicate PROJECT C have potential suppressing effects on SUDs such as alcohol, morphine and cocaine in humans.

PROJECT C (10, 30 and 100 mg/kg po) has no synergistic effect on motor coordination when combined with EtOH (0.5, 1 and 2 g/kg po), but may have antagonistic effects in some conditions of combination in a mouse accelerating rotarod performance test. GHB (0.1, 0.2 and 0.4 g/kg po) has an additive effect with EtOH. These results suggest that PROJECT C has no stronger additive interaction with EtOH compared to GHB. In the sleep EEG study, PROJECT C significantly decreased the percent REM sleep vs the total duration and the frequency of sleep interruption and significantly increased the EEG power of slow waves in non-REM sleep and the EEG power of theta waves in REM sleep in the light period in rats. These results suggest that PROJECT C may increase sleep intensity in non-REM and REM sleep. In addition, the amount of REM sleep and the EEG power have potential to be functional biomarkers.

The effect of PROJECT C on sleep EEG was investigated at 0 to 2 h after administration in the light period in rats. PROJECT C significantly decreased the REM sleep (percent REM sleep vs total duration) and the frequency of sleep interruption at an oral dose of 10 mg/kg, but did not show a significant effect on the percent non-REM sleep vs total duration and wakefulness at doses up to 10 mg/kg orally [[Figure 10](#_bookmark22)]. In addition, PROJECT C significantly increased the EEG power of delta and theta waves in non-REM sleep and the EEG power of theta waves in REM sleep at an oral dose of 10 mg/kg in rats [[Figure 11](#_bookmark23)]. Baclofen also significantly decreased the percent REM sleep vs the total duration and significantly increased the EEG power of theta waves in REM sleep at an intraperitoneal dose of 3 mg/kg in rats.

### Pharmacokinetics and Metabolism

The plasma protein binding of PROJECT C was 98.24% to 98.42% in humans. No human-specific PROJECT C metabolites were identified among those formed in liver

microsomes or hepatocytes. CYP3A4 and CYP2D6 were involved in the metabolism of PROJECT C. CYP3A4 is considered to be a major metabolizing enzyme of PROJECT C based on the higher relative abundance of CYP3A4 compared to CYP2D6 [Shimada et al, 1994]. The　IC50 values for CYP inhibition by PROJECT C were 9.25 µmol/L for CYP2C9 and > 10 µmol/L for CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6 and CYP3A. No time-dependent decrease of IC50 was observed for all isoforms tested.

### The Rationale for Animal Selection for Toxicity Studies

Sprague-Dawley rats and beagle dogs were considered appropriate animal species to assess the safety of PROJECT C since the in vitro metabolic fingerprinting study using cryopreserved hepatocytes showed the major human metabolites were covered by both rats and dogs.

### Target Organ Toxicities

The exposure levels of PROJECT C were measured in the safety pharmacology, repeated-dose toxicity studies and embryo-fetal development studies compared with exposure at steady state for 30 mg (tablet A formulation) once daily dose in Study Project C-CL-0002 [[Table 1](#_bookmark49)].

The exposures at 25 mg tablet B formulation are anticipated to provide comparable margins than those provided for 30 mg tablet A formulation.

### Table 1 Exposure Ratios of PROJECT C Based on Animal Cmax and AUC24 in the Safety Pharmacology, Repeated-dose Toxicity Studies and Embryo-fetal Development Studies Based on Steady-state Exposure at 30 mg Once Daily Dosing in Study Project C-CL-0002

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species/Study Duration (Study Number)** | **Dose (mg/kg/day)** | **Sex** | **Cmax (ng/mL)** | | **AUC24**  **(ng·h/mL)** | | **Last Dose** | |
| **Cmax** | **AUC24** |
| **First Dose** | **Last Dose** | **First Dose** | **Last Dose** | **Exposure Margin†** | |
| Dog/Telemetry (Project C-PT-0002) | 1 | M | 45 | NA | 390 | NA | 0.4 | 0.2 |
| 3 | M‡ | 92 | NA | 977 | NA | 0.8 | 0.5 |
| 10 | M | 188 | NA | 1765 | NA | 1.5 | 1.0 |
| 100 | M | 398 | NA | 4970 | NA | 3.3 | 2.7 |
| Rat/CNS  (Project C-PT-0001) | 10 | M | 158 | NA | 1064 | NA | 1.3 | 0.6 |
| 300 | M‡ | 1282 | NA | 28486 | NA | 10.5 | 15.6 |
| 2000 | M | 4104 | NA | 52583 | NA | 33.6 | 28.7 |
| Rat/4-week (Project C-TX-0001) | 10 | M‡ | 222 | 245 | 1075 | 1579 | 2.0 | 0.9 |
| F | 253 | 299 | 1147 | 1474 | 2.5 | 0.8 |
| 300 | M | 1509 | 1136 | 28124 | 14986 | 9.3 | 8.2 |
| F‡ | 1463 | 1733 | 24542 | 18665 | 14.2 | 10.2 |
| 2000 | M | 3536 | 3074 | 61629 | 39315 | 25.2 | 21.5 |
| F | 2324 | 3269 | 43651 | 36219 | 26.8 | 19.8 |
| Dog/4-week (Project C-TX-0002) | 1 | M | 78 | 105 | 630 | 845 | 0.9 | 0.5 |
| F | 72 | 76 | 499 | 543 | 0.6 | 0.3 |
| 3 | M‡ | 180 | 247 | 1725 | 2339 | 2.0 | 1.3 |
| F | 175 | 235 | 1535 | 1847 | 1.9 | 1.0 |
| 10 | M | 242 | 311 | 1426 | 3025 | 2.5 | 1.7 |
| F‡ | 279 | 436 | 3256 | 4657 | 3.6 | 2.5 |
| 100 | M | 963 | 1703 | 7620 | 21551 | 14.0 | 11.8 |
| F | 904 | 1971 | 6542 | 27895 | 16.2 | 15.2 |
| *Table continued on next page* | | | | | | | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species/Study Duration (Study Number)** | **Dose (mg/kg/day)** | **Sex** | **Cmax (ng/mL)** | | **AUC24**  **(ng·h/mL)** | | **Last Dose** | |
| **Cmax** | **AUC24** |
| **First Dose** | **Last Dose** | **First Dose** | **Last Dose** | **Exposure Margin†** | |
| Rat/13-week (Project C-TX-0007) | 10 | M | 290 | 179 | 1340 | 1483 | 1.5 | 0.8 |
| F | 277 | 251 | 1289 | 1308 | 2.1 | 0.7 |
| 100 | M‡ | 1211 | 807 | 15447 | 7929 | 6.6 | 4.3 |
| F‡ | 923 | 1346 | 13397 | 14041 | 11.0 | 7.7 |
| 2000 | M | 4731 | 2400 | 70678 | 31916 | 19.7 | 17.4 |
| F | 2153 | 2401 | 41739 | 42346 | 19.7 | 23.1 |
| Dog/13-week (Project C-TX-0008) | 3 | M‡ | 276 | 202 | 2224 | 1783 | 1.7 | 1.0 |
| F‡ | 196 | 247 | 1449 | 1865 | 2.0 | 1.0 |
| 10 | M | 566 | 505 | 4495 | 5402 | 4.1 | 3.0 |
| F | 464 | 492 | 5483 | 3697 | 4.0 | 2.0 |
| 30 | M | 771 | 466 | 8038 | 4177 | 3.8 | 2.3 |
| F | 617 | 1021 | 4286 | 7769 | 8.4 | 4.2 |
| Rat/Embryo-Fetal  Development (Project C-TX-0011) | 10 | F‡ | 354 | 531 | 1600 | 2590 | 4.4 | 1.4 |
| 300 | F | 1500 | 2400 | 25900 | 36100 | 19.7 | 19.7 |
| 2000 | F | 2080 | 3480 | 32400 | 42600 | 28.5 | 23.3 |
| Rabbit/Embryo- fetal development (Project C-TX-0013) | 10 | F‡ | 525 | 414 | 2930 | 1880 | 3.4 | 1.0 |
| 30 | F | 835 | 1120 | 7040 | 7070 | 9.2 | 3.9 |
| 100 | F | 1570 | 2640 | 20300 | 34200 | 21.6 | 18.7 |

NA: not applicable; M: male; F: female; NOAEL: no observed adverse effect level.

† Ratio comparing mean exposure (Cmax: 122 ng/mL, AUC24: 1830 ng·h/mL) determined at steady state at the dose of 30 mg (qd) in phase 1 clinical Study Project C-CL-0002.

‡ Indicates the NOAEL.

A summary of potential safety concerns from safety pharmacology, repeated-dose toxicity, genotoxicity, reproductive and developmental and phototoxicity studies and their relevance to human usage are presented in [Table 2](#_bookmark50). The anticipated maximum therapeutic dose of PROJECT C is estimated to be 25 mg once daily with newly developed tablet B formulation which is expected to show comparable exposures at 30 mg once daily with previous tablet A formulation used in phase 1 clinical studies including Project C-CL-0002.

### Table 2 Summary of Nonclinical Safety Observations and Potential Human Relevance

|  |  |  |
| --- | --- | --- |
| **Potential Safety Concern (from Nonclinical Studies)** | **Relevance to Human Usage** | **30 mg Once Daily (Steady State)** |
| GI effects  **Dog:** vomiting | Potential risk of  vomiting | Possible |
| Effects on CNS/Clinical Signs  **Rat:** ↓body temperature, ↓spontaneous activity  **Dog:** salivation, vomiting, ataxic gait, somnolence,  ↓spontaneous activity | Potential risk of vomiting, ataxia, sedation, somnolence | Possible |
| Effects on General Condition  **Rat:** ↓food consumption, ↓body weight  **Dog:** ↓food consumption, ↓body weight, 1 male sacrificed due to moribundity | Potential risk of decreased appetite, decreased body weight | Possible |
| Effects on Blood Potassium Levels  **Dog:** ↓blood potassium | Hypokalemia, QT prolongation | Not expected |
| Effects on Cardiovascular Function  **In vitro:** suppression of hERG current  **Dog:** ↓blood potassium, ↓heart rate, ↑QT/QTcM interval in 1 male | Low heart rate, QT prolongation | Not expected |
| Effects on Thyroid  **Rat:** ↑thyroid weight, hypertrophy of follicular cell | Hyperthyroidism | Not expected |
| Effects on Male Reproductive Organ  **Dog:** atrophy of bilateral seminiferous tubules in the testes, absence of sperm in the epididymides in 1 male | Male infertility | Not expected |
| Effects on Hematopoiesis  **Rat:** ↓erythrocyte count, ↓hemoglobin, ↓hematocrit  **Dog:** ↓erythrocyte count, ↓hemoglobin, ↓hematocrit in males | Anemia | Not expected |
| Effects on Thymus and Pancreas  **Dog:** atrophy of the thymus, ↓zymogen granules in the pancreas | Effects on thymus and pancreas | Not expected |
| Effects on Embryo-fetal Development  **Rat:** ↓food consumption and body weight in dams, no effects in fetuses  **Rabbit:** ↓food consumption and body weight in dams, no effects in fetuses | No effect on embryo- fetal development | Not expected |

Note: Exposure in the phase 1 and 2a studies using the tablet A formulation is the anticipated maximum therapeutic dose of PROJECT C 30 mg once daily in humans. Toxicities are not expected at the current maximal dose of 30 mg once daily; however, at higher exposures, some of the effects observed in the animal studies may occur. The anticipated maximum therapeutic dose of PROJECT C is estimated to be 25 mg once daily with the newly developed formulation (tablet B), which is expected to show comparable exposures at 30 mg once daily with the previous formulation (tablet A).

↓: decreased; ↑: increased; CNS: central nervous system; GI: gastrointestinal; hERG: human ether-à-go-go- related gene; QTcM: QT interval corrected for heart rate using Matsunaga’s correction.

### Moribundity/Mortality

One female rat at 2000 mg/kg in the 13-week study showed decreases in body weight and food consumption and was found dead on day 73 of dosing. Measured exposure level on day 91 for this group was 19.7-fold for Cmax and 23.1-fold for AUC24 higher than the exposure of once daily 30 mg at steady state (122 ng/mL for Cmax and 1830 ng·h/mL for AUC24) as determined in phase 1 Study Project C-CL-0002. In dogs, 1 male at 100 mg/kg in the 4-week study showed severe clinical signs as well as effects on food consumption and body weight and was sacrificed due to moribundity on day 14. The exposure level measured for this group on day 28 was 14.0-fold for Cmax and 11.8-fold for AUC24 higher than the clinical exposure at maximum therapeutic dose. For both rats and dogs, similar signs and changes were observed in surviving animals in the same dose group with rapid recovery by withdrawal of dosing. No related findings were observed in the phase 1 clinical studies.

### Clinical Signs Based on Excessive Pharmacological Effect

Decreased body temperature (CNS safety pharmacology study) and decrease in spontaneous activity (repeated-dose study) were noted in rats; and salivation, vomiting, somnolence, ataxic gait and decrease in spontaneous activity were noted in dogs (repeated-dose and telemetry studies). These findings may be due to an exacerbated pharmacological action of PROJECT C. Vomiting was observed at plasma concentration (telemetry study) 1.5-fold the clinical Cmax exposure at maximum therapeutic dose and other findings were observed at higher exposure. The changes were reversible. Similar observations such as salivation, emesis, ataxia, recumbence and decreased activity have been noted for GABAB receptor agonist, baclofen [GABLOFEN prescribing information, 2015].

### Decrease in Blood Potassium Levels

A decrease in blood potassium concentration was observed in dogs (4-week repeated-dose and telemetry studies) at 100 mg/kg (exposure levels were 3.3-fold for Cmax and 2.7-fold for AUC24 higher than the clinical exposure at maximum therapeutic dose). This change was not accompanied by histopathological changes in the kidneys and adrenals and was reversible. In the 13-week study in dogs conducted with the dose level of the MTD (30 mg/kg) and lower, no such decrease of potassium was noted. No related findings were observed in the phase 1 clinical studies.

### Effect on Cardiovascular System

In vitro suppression of hERG current was noted at concentrations ≥ 0.661 µmol/L

(269 ng/mL), and decreased heart rate and prolongation of QT and QTcM were noted in 1 male dog at 100 mg/kg (4-week repeated-dose study) where exposure levels on day 28 were 14.0-fold higher than the clinical Cmax exposure at maximum therapeutic dose. No

histopathological changes in the heart were noted. The reversibility of decreased heart rate and QT prolongation was not confirmed because of low incidence. QT and QTcM prolongation may be related to decreased blood potassium levels. No such changes were noted in the 13-week study in dogs conducted with the dose levels < MTD (30 mg/kg).

Similar effects were observed in the 1-week repeated-dose study of baclofen in dogs (data on file). No related findings were observed in the phase 1 clinical studies.

### Effect on Thyroid

Increased weight and hypertrophy of follicular cells in the thyroid were noted in the 4-week repeated-dose study in rats (exposure levels on day 28 were 9.3-fold for Cmax and 8.2-fold for AUC24 higher than the clinical exposure at maximum therapeutic dose). Similar changes have been reported to be induced by hepatic enzyme induction specifically in rats. In this

study, however, no evidence was found of enzyme induction such as an increase in liver weight, hepatocellular hypertrophy or exposure reduction after repeated dosing.

Subsequently, a mechanistic study conducted in rats suggested that PROJECT C has a potential to induce hepatic UDPGT, but measured activities were not increased statistically significantly in the liver from rats treated with 2000 mg/kg of PROJECT C for 1 week.

Although the mechanism of the change (follicular cell hypertrophy in the thyroid) is still not clearly identified, it was confirmed to be reversible and, in addition, was not reproduced in the 13-week toxicity study even under higher exposures and longer durations. From these data, it is not considered that PROJECT C will induce serious adverse effect on the thyroid, whether observed follicular cell hypertrophy in rats was caused by a likely hepatic enzyme induction or not. No related findings were observed in the phase 1 clinical studies.

### Effect on Male Reproductive Organ

In the dog 4-week repeated-dose study, atrophy of bilateral seminiferous tubules in the testes and absence of sperm in the epididymides were observed at 100 mg/kg (exposure on day 28 were 14.0-fold for Cmax and 11.8-fold for AUC24 higher than the clinical exposure at maximum therapeutic dose). Despite low incidence (1 male), causal relationship with the drug could not be ruled out because of the severe grade of the findings. The reversibility of these findings was not confirmed due to the low incidence. At this dose level of 100 mg/kg, another male was sacrificed in moribundity and 100 mg/kg of PROJECT C was considered to exceed the MTD. Actually, the changes in testes and epididymis were observed in an animal accompanied by a marked decrease in body weight, by more than 15% of the initial value at maximum, and the changes could have been due to such a severe body weight loss. In the dog 13week study that was subsequently conducted with dose levels of the MTD

(30 mg/kg) and lower, no abnormal findings were observed in the testes or epididymides. Considering that bilateral atrophy of seminiferous tubules in the testes and absence of sperm in the epididymides were observed in 1 male dog at 100 mg/kg, this finding is not expected to occur at the proposed dose levels in human.

### Effect on Hematopoiesis

Some erythrocyte parameters fluctuated in the repeated-dose studies in rats and dogs. Although decreased reticulocyte ratio, hypocellularity in bone marrow, decreased weight and extramedullary hematopoiesis in spleen were noted in a preliminary 1-week repeated-dose study in rats, no histopathological changes in the bone marrow, spleen and liver were noted at 2000 mg/kg in the 4- and 13-week repeated-dose studies. In the 4-week repeated-dose study in dogs, males showed decreased erythrocyte parameters (exposure on day 28 were 14.0-fold for Cmax and 11.8-fold for AUC24 higher than the clinical exposure at maximum therapeutic dose) and females showed hemoconcentration (exposure on day 28 were 16.2-fold for Cmax and 15.2-fold for AUC24 higher than clinical exposure at maximum therapeutic dose). Since anemic changes were observed at dose levels that were lethal for some of the animals treated and clinical signs and decreases in body weight and food consumption were noted in both species, it was unclear if the hematopoietic system is a toxicological target of PROJECT C or not. This anemic change was confirmed to be reversible and no such changes were noted at

the MTD (100 mg/kg in rats, 30 mg/kg in dogs) and lower dose levels in the 13-week studies. Therefore, it is unlikely that the clinical dose range of PROJECT C would induce serious anemia in humans. No related findings were observed in the phase 1 clinical studies.

### Effect on Thymus and Pancreas

Atrophy of the thymus and decreased zymogen granules in the pancreas were noted in the 4-week repeated-dose study in dogs. The findings were considered to be stress responses because they were observed in a group of dogs exhibiting marked decrease in food consumption and body weight. The findings were reversible. No related findings were observed in the phase 1 clinical studies.

### Effect on Embryo-fetal Development

In the preliminary embryo-fetal development study in rats, decrease in body weight and low number of ossified sacral and caudal vertebrae in fetuses were observed. Those findings were considered to be secondary to generalized growth retardation of fetuses related to the maternal toxicity at 2000 mg/kg rather than a direct evidence of teratogenicity of PROJECT C. In the embryo-fetal development study in rats and rabbits, PROJECT C was not teratogenic, but did exhibit maternal toxicity (decreases in body weight and food consumption). The NOAEL was 10 mg/kg for dams in rats and rabbits, and was 2000 mg/kg and 100 mg/kg for fetuses in rats and rabbits, respectively.

### Interaction with Opioids

In overdose settings, respiratory depression is known to be induced by buprenorphine (when taken with benzodiazepines or alcohol) and morphine, a partial and full agonist for the opioid μ receptor, respectively. PROJECT C was confirmed to have very low binding affinity toward the opioid μ receptor at 10 μmol/L (4076 ng/mL; 16.5-fold higher than the mean exposure limit in clinical studies), indicating a pharmacodynamic interaction via the opioid μ receptor is unlikely. Given that morphine, a full opioid μ receptor agonist, has a larger magnitude of effect on respiratory depression than buprenorphine, an in vivo study with PROJECT C and morphine was conducted. In this study, morphine-induced respiratory depression was not potentiated by PROJECT C and there was no apparent abnormality in behavior of the animals, even after concomitant administration of PROJECT C and morphine at supratherapeutic doses (10 mg/kg for both PROJECT C and morphine). Respiratory depression is also reported to be potentiated by benzodiazepines when concomitantly administered with opioids [Kim et al, 2017]; however, PROJECT C was confirmed to have negligible binding affinity to the benzodiazepine-binding site of the GABAA receptor.

The pharmacokinetic DDI potential of PROJECT C with opioids (i.e., morphine, buprenorphine, hydromorphone, codeine, oxycodone, hydrocodone, fentanyl and methadone), naloxone, ethanol and cocaine were assessed based on the relevant data of PROJECT C, literature and regulatory guidance [FDA Drug Interaction Studies Guidance for Industry, 2020; MHLW Guideline on Drug Interactions, 2018; EMA Guideline on the Investigation of Drug Interactions, 2012]. PROJECT C is mainly metabolized by CYP3A4 as suggested by an in vitro CYP study. The IC50 values of PROJECT C were 9.25 µmol/L for CYP2C9 and > 10 µmol/L

for all other CYP isoforms tested. In addition, PROJECT C did not show more than dose proportional pharmacokinetics or time variant pharmacokinetics in humans. These data suggest that there is no significant inhibitory effect of PROJECT C for CYPs under the proposed clinical dose/exposure. Neither opioids, naloxone, ethanol nor cocaine is listed as a sensitive substrate nor as an inhibitor or inducer of CYP3A4 in the regulatory guidances above.

Furthermore, these substances are reported to be metabolized via multiple enzymes. Therefore, a DDI between PROJECT C and these substances is considered to be unlikely.

In the toxicology studies of PROJECT C, toxicity findings observed at the lowest-observed- adverse-effect level were vomiting and salivation in dogs, at the exposure levels relatively close to the mean exposure limit in clinical studies. Vomiting and salivation were also reported in the repeated dose studies in dogs of buprenorphine and buprenorphine/naloxone (FDA Pharmacology reviews for Subutex and Suboxone) and opioid receptors, particularly μ receptor, have been suggested to be involved in opioid-induced vomiting [Porreca & Ossipov, 2009]. Opioid μ receptor does not appear to be involved in the vomiting observed by PROJECT C, given the affinity of PROJECT C to the opioid μ receptor as mentioned above.

Vomiting and salivation in PROJECT C, buprenorphine and buprenorphine/naloxone were reversible and considered to be monitorable and manageable in clinical studies. Vomiting was not observed in monkeys administered PROJECT C and morphine at supratherapeutic levels.

### Conclusion

Taken together, changes caused by exposure to PROJECT C consisted of clinical signs such as vomiting, salivation, somnolence, ataxic gait, decreased spontaneous activity, decreased food consumption, decreased body weight, decreased blood potassium levels, effects on the cardiovascular system (decreased heart rate and QTcF prolongation), effects on the thyroid, effects on the thymus, effects on the pancreas, male reproductive organ toxicity and effects on hematopoiesis. Potential safety concerns of PROJECT C are summarized in [Table 2.](#_bookmark50)

In conclusion, data from the nonclinical studies suggest that PROJECT C may have therapeutic potential as a treatment for AUD and OUD mainly through its positive allosteric modulating action on the GABAB receptor. There were no pharmacokinetics or metabolism concerns preventing clinical study and none of the toxicology findings would prevent continuation of clinical studies with treatment durations of up to 13 weeks.

#### List of References

GABLOFEN (prescribing information). Hazelwood, MO: Mallinckrodt Brand Pharmaceuticals, Inc.; February 2015.

Kim HS, McCarthy DM, Mark Courtney D, Lank PM, Lambert BL. Benzodiazepine-opioid co-prescribing in a national probability sample of ED encounters. Am J Emerg Med. 2017;35:458-64.

Porreca F and Ossipov MH. Nausea and vomiting side effects with opioid analgesics during treatment of chronic pain: mechanisms, implications, and management options. Pain Med.

2009;10:654-62.

Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther. 1994;270:414-23.