### Safety Pharmacology

An overview of safety pharmacology studies is provided in End-of-Text Table 1.2.

### Effects on the Cardiovascular System

The effects of PROJECT O on the human ether-a-go-go-related gene (hERG) current were studied in hERG-transfected HEK293 cells by the whole-cell patch-clamp technique (Study Project O-PT-0001). The compensated suppression rates of PROJECT O at the concentrations of 0.610 x 10-7, 1.98 x 10-7 and 0.674 x 10-6 mol/L were -4.6%, -6.9% and4.5%, respectively. No statistically significant difference was noted at these 3 test concentrations when compared to the rate in the control group. These results indicate that PROJECT O does not affect the hERG current in hERG-transfected HEK293 cells at concentrations of up to 0.674 x 10-6 mol/L (approximately 492 ng/mL).

The effects of PROJECT O on action potentials in isolated guinea-pig papillary muscles were studied by the glass-electrode technique under a surface-superfusing condition

(Study Project O-PT-0004). When PROJECT O was applied for 45 minutes at the concentration of

1.99 x 10-7 mol/L, the percent changes in action potential duration (APD)30, APD90 and APD30-90 were 0.2%, 1.6% and 4.7%, respectively. No effects were noted on the APD. On the resting membrane potential, action-potential amplitude or dV/dtmax, no effects were noted, either. The results indicate that PROJECT O does not affect the action potentials in isolated guinea-pig papillary muscles at the concentration of 1.99 x 10-7 mol/L (145 ng/mL).

In male beagle dogs under unanesthetized conditions administered a single oral dose of PROJECT O at dose levels of 3, 10 and 30 mg/kg at a dose volume of 5 mL/kg, PROJECT O did not affect the blood pressure, heart rate or electrocardiogram parameters such as QRS duration or PR, QT or corrected QT interval using Fridericia’s formula

(Study Project O-PT-0003). These results indicate that PROJECT O has no effect on the cardiovascular system at doses of up to 30 mg/kg.

### Effects on the CNS

In order to investigate the effects of a single dose of PROJECT O at dose levels of 10, 30 and 100 mg/kg on the CNS, the effects on the general activity and behavior in male rats were determined (Study Project O-PT-0002). PROJECT O at the dose of 10, 30 or 100 mg/kg did not affect the general activity and behavior in any of rats up to 24 h after administration. The results indicate that PROJECT O has no effect on the CNS at doses of up to 100 mg/kg.

In male beagle dogs under unanesthetized conditions administered a single oral dose of PROJECT O at dose levels of 3, 10 and 30 mg/kg at a dose volume of 5 mL/kg, PROJECT O did not affect the body temperature (Study Project O-PT-0003). During the observation of general activity and behavior, transient vomiting after administration of PROJECT O 10 and 30 mg/kg was noted in 1 and 3 animals, respectively.

### Effects on the Respiratory System

In male beagle dogs under unanesthetized conditions administered a single oral dose of PROJECT O at dose levels of 3, 10 and 30 mg/kg at a dose volume of 5 mL/kg, PROJECT O did not affect respiration rate, blood gases (blood pH, PaO2, PaCO2, SaO2) or blood-electrolyte concentrations (sodium, potassium, chloride, calcium) (Study Project O-PT-0003). These results indicate that PROJECT O has no effect on the respiratory system at doses of up to 30 mg/kg.

### Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction studies have been conducted.Other Pharmacology Studies

No other pharmacology study was conducted.

## Toxicology

An overview of toxicology studies is provided in End-of-Text Table 3.1.

### Single-dose Toxicity

Noteworthy findings from single-dose toxicity studies are presented in End-of-Text Table 3.4.

PROJECT O was administered orally as a single dose at dose levels of 100, 200 and 400 mg/kg to 5 male and 5 female SD rats (Study Project O-TX-0004). Clinical signs, body weight, food consumption and gross pathology examinations were conducted and histopathological evaluation was performed on the organ showing grossly visible indications of morphologic abnormalities. The high dose level was selected based on the MFD concentration of

20 mg/mL for soluble vehicle, 10% hydroxypropyl-β-cyclodextrin (HP-β-CD) containing propylene glycol (PG) solvent and the maximum acceptable dose volume of 20 mL/kg for a single dose in rats. Because of the specific vehicle, the negative control and vehicle control were designated as group 1 (water for injection) and group 2 (soluble vehicle: 10% HP-β-CD containing PG solvent), respectively.

A tendency for decreased food consumption was observed in males and females in 100, 200 and 400 mg/kg groups on day 1 when compared to the control group

(Study Project O-TX-0004).

No test substance-related effects on body weight and necropsy were observed in any animals in any dosing groups.

Based on the results of Study Project O-TX-0004, PROJECT O was well-tolerated up to 400 mg/kg (MFD) without remarkable toxicity when administered orally to rats.PROJECT O was administered orally as a single dose at dose levels of 150 and 300 mg/kg to male and female beagle dogs (1/sex/group) (Study Project O-TX-0005). Clinical signs, body weight, food consumption, hematology, blood chemistry and toxicokinetics were conducted. The high dose level was selected based on the MFD concentration of 20 mg/mL and the maximum acceptable dose volume of 15 mL/kg for a single dose in dog. Because of the specific vehicle, the negative control and vehicle control were designated as group 1 (water for injection) and group 2 (soluble vehicle: 10% HP-β-CD containing PG solvent), respectively.

There were no deaths in any animals in any dosing groups during the study (Study Project O-TX-0005).

Vomiting containing the test substance was observed in males and females at 150 and

300 mg/kg at 20 to 50 minutes after dosing. Also, salivation was observed in the female at 300 mg/kg from 20 minutes to 1 h postdose. These changes were not observed in the test substance groups at 2 h and thereafter. Vomiting was also seen in females of the water and vehicle control groups, but it was only a small volume of foam.

No test substance-related toxicity effects on body weights, food consumption and hematology were evident in any animals in any dosing groups.

In blood chemistry, total bilirubin (T-bil), direct bilirubin (D-bil), indirect bilirubin (I-bil) and TBA were increased in male and female at 300 mg/kg on day 1. High value of T-bil at

300 mg/kg was mainly due to the high value in D-bil. At 150 mg/kg, an increase of TBA in female was only evident on day 1, and no remarkable changes were observed in the bilirubin values in both sexes and TBA in male. There were no increases in these parameters on days 7 and 14.

In toxicokinetics, vomiting, which included detectable levels of test article-like substance, occurred in both male and female dogs within 1 hour post-dose; meaningful increases in Cmax and AUC24 did not occur consistently across the dose range from 150 to 300 mg/kg

(Study Project O-TX-0005).

Based on the results of Study Project O-TX-0005, a single oral administration of PROJECT O was well tolerated up to 300 mg/kg in dogs with transient clinical signs of vomiting and salivation after dosing and increases in T-bil, D-bil, I-bil and TBA on day 1 after dosing.

### Repeat-dose Toxicity

Tabulated data for pivotal repeat-dose toxicity studies are presented in End-of-Text Table 3.6.

Definitive 4-week oral repeated dose toxicity studies of PROJECT O were conducted in both rats and dogs with PROJECT O (free form, development form).

### Rat

* + - * 1. **4-week Repeated Oral Dose Toxicity Study**

PROJECT O dissolved in the vehicle, soluble vehicle: 10% HP-β-CD containing PG solvent in order to maximize systemic absorption and exposure without local irritation wasadministered orally once daily for 4 weeks at dose levels of 3, 10, 30 and 100 mg/kg to 10 male and 10 female Crl:CD(SD) rats per group in order to investigate its toxicity

(Study Project O-TX-0006). The highest dose level was set at 100 mg/kg per day based on the MFD concentration of 20 mg/mL and the maximum acceptable dose volume of 5 mL/kg for the vehicle. Five males and 5 females were added to the negative and vehicle control groups and the 100 mg/kg group to assess the reversibility of toxicity observed during the dosing period in a subsequent 4-week recovery period. A satellite group (3 males and 3 females in each control group and 12 males and 12 females in each test-article group) was added at each dose level to assess systemic exposure to PROJECT O. The following observations and examinations were performed in this study: clinical signs, body weight, food consumption, ophthalmology, urinalysis, hematology, blood chemistry, PTH analysis, gross pathology, organ weights, histopathology and toxicokinetics.

No animal died in any group. No toxic changes were noted in any group in any examination during the dosing or recovery period (Study Project O-TX-0006).

Low urinary inorganic phosphorus excretion was noted in females in the 10 mg/kg group and above at week 2 of dosing, whereas there were no clear changes in urinary inorganic phosphorus excretion at week 4 of dosing or serum inorganic phosphorus level at the end of the dosing period. Low urinary inorganic phosphorus excretion was considered to be related to the pharmacological effect (inhibition of inorganic phosphate absorption in the small intestine) of the test article; therefore, the change was not considered to be toxicologically significant.

In the vehicle control group, slightly high aspartate transaminase and alanine transaminase and slightly high adrenal weight were noted in both sexes, but no corresponding histopathological findings were observed in the liver or adrenal. This slightly elevated transaminase is considered to be caused by HP-β-CD, component of the vehicle [Thackaberry et al, 2010; Gould & Scott, 2005]. Statistically significant high absolute adrenal weight was also noted in males and high relative adrenal weight was noted in females.

In toxicokinetics, Cmax and AUC24 generally increased less than dose proportionally in both sexes and there were no sex differences or repeated dose effects in any toxicokinetic parameter during the dosing period.

It was concluded that, under the conditions of Study Project O-TX-0006, the NOAEL was 100 mg/kg per day for males and females.

### Dog

* + - * 1. **4-week Repeated Oral Dose Toxicity Study**

PROJECT O dissolved in the soluble vehicle, 10% HP-β-CD containing PG solvent, was administered orally once daily for 4 weeks at dose levels of 3, 10, 30 and 100 mg/kg to 4 male and 4 female beagle dogs per group in order to investigate its toxicity (Study Project O-TX-0007). The highest dose level was set at 100 mg/kg per day based on the MFD concentration of 20 mg/mL and the maximum acceptable dose volume of 5 mL/kg for the vehicle. Three males and 3 females were added to the 100 mg/kg group in order to assess the reversibility of toxicity during a subsequent 4-week recovery period.

The animals in the control groups received water for injection (negative control) or the vehicle (vehicle control). Systemic exposure to PROJECT O was also evaluated. The following observations and examinations were performed: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, gross pathology, organ weights, histopathology, electron microscopy and PTH analysis.

Soft stool was observed in all test article groups at the same frequency as in the vehicle control group, and this symptom was considered as an effect of the vehicle.

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

At 10 mg/kg, plasma ALP and T-bil increased or tended to increase except for ALP in females, albumin and total protein exhibited decreasing trends in some male and female animals. Hypertrophy and proliferation of Kupffer cells in the liver (slight or very slight) were observed in 1 male and all females.

At higher dose levels (30 and 100 mg/kg), vomiting was observed in all males and all females, mainly 1 h after dosing. Slight decreases were noted in body weight in males and females and in food consumption in females at 100 mg/kg. The following blood chemistry and histopathological changes were observed in a dose-dependent manner: increased ALP and TBA in males at 30 mg/kg and more and in females at 100 mg/kg; increased T-bil (mainly due to increased D-bil) and decreased albumin in males and females at 30 mg/kg or more; decreased calcium in males at 30 mg/kg and greater; decreased total protein in males at 100 mg/kg and in females at 30 mg/kg and greater; and hypertrophy and proliferation of the Kupffer cells (moderate or marked), mononuclear cell infiltration (very slight or slight) and/or very slight clear cell change in the centrilobular hepatocytes in the liver in males and females at 30 mg/kg and greater. In the Kupffer cells, cytoplasm was positive for periodic acid-Schiff stain. Under electron microscopy, the Kupffer cells at 100 mg/kg per day contained enlarged irregularly shaped lysosomes including amorphous/fibrillary/laminated structures.

Due to their location in the sinusoids of the liver and their nature (fixed macrophages), Kupffer cells are the first cells of the mononuclear phagocyte system to come into contact with foreign materials coming from the gastrointestinal tract [Racanelli & Rehermann, 2006]. Although no histopathological changes were noted in the bile duct, above-mentioned blood chemistry and histopathological findings were indicative of some hepatobiliary effect of PROJECT O. At the end of the 4-week recovery period, hypertrophy and proliferation of the Kupffer cells in the liver remained, but the incidence and severity of these changes were lower than those at the end of the dosing period. Other changes which had been caused by 4-week treatment completely recovered.

In toxicokinetics, on day 1, tmax did not differ between dose levels. Cmax and

AUC24 increased dose-dependently, but less than dose proportionally, at all dose levels except in females at 100 mg/kg. Cmax and AUC24 in females at 100 mg/kg were lower than those at 30 mg/kg. Vomiting was observed in a number of animals at 100 mg/kg. Although the amount of administered test article expelled in vomitus was unclear, the loss was considered one of the reasons for the low exposure. After repeated dosing, Cmax and AUC24 did not change at 3 to 30 mg/kg. At 100 mg/kg, these parameters increased with repeated dosing, and a dose-proportional or slightly greater than dose-proportional increase in exposure (AUC24) from those at 3 mg/kg was noted at 100 mg/kg in males on days 14 and 28 and in females at day 28. After repeated dosing, tmax did not differ. There were no clear sex differences in any parameter.

It was concluded that, under the conditions of Study Project O-TX-0007, the NOAEL was 3 mg/kg per day for males and females. Reversibility of the test article-related changes was indicated at the end of the 4-week recovery period.

### Genotoxicity

Tabulated data from in vitro genotoxicity studies are provided in End-of Text Table 3.7. The potential genotoxicity of PROJECT O was evaluated in vitro.

In order to assess the potential of PROJECT O to induce gene mutation, a bacterial reverse mutation test was performed with 5 test strains of bacteria (Salmonella typhimurium [TA100, TA1535, TA98, and TA1537] and Escherichia coli [WP2uvrA]), using the preincubation method with and without metabolic activation (Study Project O-TX-0008). Based on the results of the dose-finding test at 15, 50, 150, 500, 1500 and 5000 µg/plate, the main test was

performed at 15.6, 31.3, 62.5, 125, 250 and 500 µg/plate.

Test article precipitation was observed at 500 μg/plate and greater without metabolic activation and at 250 µg/plate and greater with metabolic activation after incubation for 48 h.

Growth inhibition was not observed up to 5000 µg/plate in any test strain with or without metabolic activation.

In comparison with the negative control, no 2-fold or greater and dose-dependent increase in the number of revertant colonies was observed in any test strain either in the dose-finding test or the main test with or without metabolic activation.

It was concluded that PROJECT O did not induce gene mutation in bacteria.

In order to evaluate whether PROJECT O induces chromosomal aberrations, a chromosomal aberration test was performed with cultured mammalian cells (CHL/IU) in short-term treatments for 6 h with and without metabolic activation, and continuous treatment for 24 h without metabolic activation (Study Project O-TX-0009).

The dose levels for the chromosomal aberration test were set based on the results of the dose-finding test. The highest dose for chromosomal analysis was set based on the cell proliferation ratio and 2 lower doses were set for chromosomal analysis. Chromosomal aberrations were analyzed at the following doses: 222, 333 and 500 µg/mL in short-term treatment with and without metabolic activation and 65.8, 148 and 222 µg/mL in continuous

treatment for 24 h. The number and incidence of cells with structural and numerical chromosomal aberrations were investigated.

Test article precipitation in the treatment medium was observed at 222 µg/mL and greater at the start and end of treatment.

The cell proliferation ratio determined from the population doubling showed dose-dependent decreases in continuous treatment for 24 h only.

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

It was concluded that, PROJECT O did not induce chromosomal aberrations in CHL/IU cells, regardless of the presence or absence of metabolic activation, or treatment length.

### Carcinogenicity

No longer-term studies have yet been conducted with PROJECT O, but the genotoxicity data shows no reason for concern in the in vitro situation.

### Reproductive and Developmental Toxicity

Tabulated data from pivotal reproductive and developmental toxicity studies are provided in End-of-Text Tables 3.11 and 3.12.

A study to assess the effects of PROJECT O on fertility and early embryonic development to implantation was conducted in rats. Studies to assess the teratogenic potential of PROJECT O were conducted in rats and rabbits.

### Effects on Fertility and Early Embryonic Development to Implantation

* + - * 1. **Study in Rats**

Tabulated data from the reproductive and developmental toxicity study on early embryonic development to implantation are provided in End-of-Text Table 3.11.

PROJECT O was administered orally to 20 male and 20 female Crl:CD(SD) rats in each group at dose levels of 0 (water for injection), 0 (10% HP-β-CD containing PG solvent), 10, 30 and 100 mg/kg per day from before mating (males and females) and through the mating period (males and females) until implantation (day 7 of gestation, females) (Study Project O-TX-0018). In females, effects on estrous cycle, fertilization, tubal transport, implantation and embryonic development at the preimplantation stage were evaluated. In males, effects on functional aspects (e.g., on libido and insemination) that might not be detected by histological examination of the male reproductive organs were evaluated. Systemic exposure to PROJECT O was also assessed.

### Effects on General Toxicity of Parental Animals

No animals died and no abnormalities were observed in clinical signs in any group. No test article-related changes were noted in body weight, body weight gain or food consumption in any test article group. No gross pathological abnormalities were observed in any group.

### Effects on Reproductive Function

No test article-related changes were noted in the frequency of estrus, estrous interval, copulation rate, mean copulatory interval, fertility rate, number of corpora lutea or number of implantations or preimplantation loss rate in any test article group.

### Effects on Early Embryonic Development

No test article-related changes were noted in the number of live embryos, number of postimplantation losses or postimplantation loss rate in any test article group.

### Toxicokinetics

Cmax and AUC24 values increased less than dose-proportionally on day 1 of dosing and following repeated administration (day 28 of dosing for males and day 14 of dosing for females) and were almost constant on both stages in both sexes. For both stages in both sexes, tmax values were 0.5 to 1 h and those values were almost constant at all doses and dosing times. There was no sex difference.

Based on these results, it was concluded that, under the conditions of Study Project O-TX-0018, the NOAEL of PROJECT O was 100 mg/kg per day for general toxicity and reproductive function in parental animals and for early embryonic development.

### Effects on Embryo-fetal Development

Tabulated data from the reproductive and developmental toxicity studies on embryo-fetal development are provided in End-of-Text Table 3.12.

### Study in Rats

PROJECT O was administered orally to 18 to 20 pregnant Crl:CD(SD) rats per group at dose levels of 0 (water for injection), 0 (10% HP-β-CD containing PG solvent), 30, 100 and 200 mg/kg per day during the period from implantation to closure of the hard palate (from

day 7 to day 17 of gestation) to investigate the effects of PROJECT O on pregnant females and embryo-fetal development (Study Project O-TX-0011). Systemic exposure to PROJECT O in dams was also assessed.

### Effect on Dams

No dams died in any group and no abnormalities were observed in clinical signs in any group. In the 200 mg/kg group, low food consumption was noted during the dosing period. No test article-related changes were noted in food consumption in the 100 or 30 mg/kg group or in body weight, body weight gain, gross pathology, number of corpora lutea or number of implantations in any test article group.

### Effect on Fetuses

No test article-related changes were noted in the number of live fetuses, number of embryofetal deaths, postimplantation loss rate, fetal body weight, placental weight, sex ratio or external or placental findings in any test article group or in visceral or skeletal findings in the 200 mg/kg group.

### Toxicokinetics

Cmax and AUC24 values increased with increasing dose on both days 7 and 17 of gestation, except Cmax on day 17 of gestation in the 200 mg/kg group. For both stages, tmax values were

0.5 to 1 h. Those values were almost constant regardless of the frequency of dosing.

Based on these results, it was concluded that, under the conditions of Study Project O-TX-0011, the NOAEL of PROJECT O was 100 mg/kg per day for dams and 200 mg/kg per day for embryo-fetal development.

### 4.3.5.2.2 Study in Rabbits

PROJECT O was administered orally to 17 to 19 pregnant Kbl:NZW rabbits per group at dose levels of 0 (water for injection), 0 (10% HP-β-CD containing PG solvent), 5, 10 and

20 mg/kg per day during the period from implantation to closure of the hard palate (from day 6 to day 18 of gestation) to investigate the effects of PROJECT O on pregnant females and embryofetal development (Study Project O-TX-0014). Systemic exposure to PROJECT O in dams was also assessed.

### Effect on Dams

No dams died in any group and no test article-related changes were noted in clinical signs, body weight, body weight gain, food consumption, gross pathology, number of corpora lutea or number of implantations in any test article group.

### Effect on Fetuses

No test article-related changes were noted in the number of live fetuses, number of embryofetal deaths, postimplantation loss rate, body weight, placental weight, sex ratio or external, placental or visceral findings in any test article group, or in skeletal findings in the 20 mg/kg group.

### Toxicokinetics

Cmax and AUC24 values increased almost dose-proportionally but slightly less than

dose-dependently at up to 20 mg/kg after the first and final dosing. For both stages, tmax values ranged from 4.0 to 6.7 h. Those values were almost constant regardless of the frequency of dosing.

Based on these results, it was concluded that, under the conditions of Study Project O-TX-0014, the NOAEL of PROJECT O was 20 mg/kg per day for dams and embryo-fetal development.

### Local Tolerance

No local tolerance studies were conducted.

### Other Toxicity Studies

No other toxicity study was conducted.

## Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

PROJECT O is under development for the treatment of hyperphosphatemia. PROJECT O is a NPT-IIb inhibitor with IC50 values of 7.0 and 88 nmol/L against human and rat NPT-IIb, respectively. In vivo studies in rats suggest that PROJECT O inhibits phosphate absorption directly in the intestinal tract and not by systemic exposure. In an adenine-induced CKD rat model with hyperphosphatemia (ASHP rats), PROJECT O decreases the elevated plasma inorganic phosphorus level. ASHP rats are a well-established model mimicking clinical complications of late-stage CKD patients. Treatment effects of vitamin D and phosphate binders on hyperphosphatemia, secondary hyperparathyroidism and vascular calcification

were clearly demonstrated in this model in the literature [Terai et al, 2009; Terai et al, 2008]. Therefore the beneficial effects of PROJECT O observed in this model can support the development of PROJECT O for the indication of hyperphosphatemia in CKD patients on dialysis.

In a screening panel, PROJECT O inhibits the radioligand binding to the CCK-A receptor by more than 50% (81.17%) at 10 µmol/L with an IC50 value of 652 nmol/L, which is much higher than that of NPT-IIb inhibition, suggesting the effect on the human CCK-A receptor is much less potent relative to the observed human NPT-IIb inhibition.

The preclinical toxicity profile of PROJECT O has been evaluated using solubilizing solvent under maximized exposure conditions in rats and dogs. All findings were evaluated for the assessment of relevance to human risk and impact on clinical trial design. Findings of potential concern for clinical trials include hepatobiliary effects.

### Target Organ Toxicity

Assessment of the safety profile suggests that increase in (direct) bilirubin, ALP and vomiting are potential human adverse responses. This identifies the liver as a potential target organ for PROJECT O. There are no indications for genotoxicity or effects on fertility or embryonic development for PROJECT O.

### Liver

The liver was identified as the main target organ of toxicity based on observations in the dog. Single and repeated dose toxicity studies in dogs [End-of-Text Tables 3.4 and 3.6.2] showed increases in total and conjugated bilirubin, ALP and TBA. These observations occurred in either or both the single dose study at the dose of at least 150 mg/kg and the 4-week repeated dose study at doses of at least 10 mg/kg. At 10 mg/kg, increased ALP was observed in

1 male; and at 30 mg/kg, T-bil, mainly D-bil, was increased in 1 male and 1 female. TBA were increased at 30 mg/kg in 2 males. At 100 mg/kg, these parameters reached statistical significance. The changes were limited to the hepatobiliary system since there were no changes in hepatocellular injury markers such as alanine aminotransferase, deposition of hemosiderin, accumulation of bile pigment or histopathological changes in the bile duct.

At 10 mg/kg and above, a dose dependent increase in hypertrophy and proliferation of Kupffer cells were observed. This change defines the NOAEL at 3 mg/kg. At 30 mg/kg per

day or more, mononuclear cell infiltration and clear cell change in the centrilobular hepatocytes occurred. Under electron microscopy, the Kupffer cells at 100 mg/kg per day contained enlarged irregularly shaped lysosomes containing amorphous/fibrillary/laminated structures. The nature of the substance observed in lysosomes is unclear.

The changes related to the liver showed the ability to recover after 4 weeks of discontinuation of PROJECT O. The findings in the dog indicate hepatobiliary changes as a potential human risk.

### Effects on Vomiting

At 30 and 100 mg/kg, vomiting was observed in all dogs, typically 1 hour after dosing, indicating limited palatability of the dosing formulation at these doses. However, it cannot be excluded that vomiting may be also a human adverse response.

### Exposure Assessment

A summary of total plasma concentrations and AUC in the 4-week repeated dose toxicology studies is presented [in Table 2.](#_bookmark60) Unbound fraction of the drug is very low (unbound fraction:

< 0.0001 for rats and 0.0004 for dogs) (Study Project O-ME-0006).

### Table 2 Animal Exposure of Total PROJECT O at the Last Day of Treatment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species/ Study Duration** | **Dose** | **Sex (M/F)** | **Cmax (ng/mL)** | **AUC24.total (ng·h/mL)** |
| Rat/4-week po | 100 mg/kg†  (NOAEL) | M | 3390.22 | 7022.18 |
| F | 3146.44 | 8646.21 |
| Dog/4-week po | 3 mg/kg  (NOAEL) | M | 293.83 | 537.98 |
| F | 456.64 | 870.05 |
| 10 mg/kg (LOAEL) | M | 1276.26 | 1971.82 |
| F | 1043.28 | 1345.33 |

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level; po: oral

† Highest dose tested.

Source: Studies Project O-TX-0006 and Project O-TX-0007

### Clinical Relevancy and Biomarkers

The nonclinical safety pharmacology and toxicology program of PROJECT O has been conducted to determine and characterize the safety profile of PROJECT O. No toxicological findings of potential concern for human risk assessment were observed except for the effects on liver and vomiting in dogs [[Table 3](#_bookmark62)]. Both could occur in humans; however, if they do occur, they would only be expected at the top of the dose range. To further characterize possible effects on the liver, serum bile acids will be added to the liver panel in the study.

### Table 3 Summary of Potential Safety Concerns

|  |  |  |
| --- | --- | --- |
| **Target Organs** | **Potential Risks** | **Risk Minimization Action** |
| Gastrointestinal Tract | Vomiting (based on dogs) | Observation |
| Liver | Increases in total bilirubin and ALP, slight hypertrophy and proliferation of the Kupffer cells in  4-week dog study, affinity for CCK-A receptor | Monitoring of liver enzymes, especially bilirubin, ALP and  bile acids |

ALP: alkaline phosphatase; CCK-A: cholecystokinin A

### Conclusions

The current nonclinical safety program supports the phase-1 studies of PROJECT O.

No critical toxicological findings of potential concern for human risk assessment were observed in the toxicology package studies except for the effects on the liver and vomiting in dogs. The above findings observed were not so serious, considered to be reversible and monitorable.

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