#### Safety Pharmacology

A total of 4 safety pharmacology studies were performed in accordance with Good Laboratory Practice (GLP) and guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). All doses and plasma concentrations are shown as PROJECT P (free base). An overview of safety pharmacological studies of PROJECT P can be found in End-of-Text Table 1.2.

#### In Vivo Effects on Central Nervous System

PROJECT P, as a single dose, was administered orally at dose levels of 50, 300, and 2000 mg/kg to male rats (Study Project P-PT-0001). PROJECT P had no effect on the general physical condition or behavior in male rats.

#### In Vitro Effects on Human Ether-à-go-go-related Gene (hERG) Current

The effects of PROJECT P on the hERG current were studied in hERG transfected human embryonic kidney (HEK293) cells using the whole-cell patch-clamp technique (Study

Project P-PT-0002). The compensated suppression rates of PROJECT P at exposure concentrations of 2.3×10−7, 2.2×10−6, and 2.5×10−5 mol/L were 6.8%, 4.0%, and 11.8%, respectively; a statistically significant difference was noted at the highest concentration of 2.5×10−5 mol/L when compared to the rate in the control group.

#### In Vitro Effects on Action Potential Duration

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

(Study Project P-PT-0003). The test solutions of PROJECT P prepared at concentrations of 3×10−7, 3×10−6, and 3×10−5 mol/L were applied for 35 min onto isolated papillary muscles in each experimental group. In the PROJECT P groups, no effects on action potential duration, resting membrane potential, action potential amplitude or the maximum rate of change of volts per second (dV/dt max) were observed at any concentration.

#### In Vivo Effects on Central Nervous, Cardiovascular and Respiratory Systems

PROJECT P, as a single dose, was administered orally at dose levels of 20, 100, and

1000 mg/kg to male dogs (Study Project P-PT-0004). PROJECT P did not affect the general activity and behavior, body temperature, blood pressure, heart rate, electrocardiogram, respiration rate, blood gases, or blood-electrolyte concentration at the dose of 20 mg/kg. At 100 mg/kg, salivation was noted in 1 animal. At 1000 mg/kg, salivation in 1 animal, vomiting in

3 animals, grayish stool in 2 animals, compound-colored feces in 2 animals were observed. A statistically significant increase in heart rate (up to 25 beats/min increase from pre-dosing) was noted 2 to 8 h after administration in animals treated with 1000 mg/kg PROJECT P.

## Toxicology

A total of 12 toxicology studies were conducted in vitro and in rats, dogs and rabbits as part of the PROJECT P development program. All pivotal studies were performed in accordance

with GLP and ICH guidelines. All doses and plasma concentrations are shown as PROJECT P (free base). An overview of toxicology studies of PROJECT P can be found in End-of-Text Tables 3.1, 3.2 and 3.3.

#### Single-dose Toxicity

Tabulated results of single-dose toxicity studies can be found in End-of-Text Table 3.4.

#### Single-dose Oral Toxicity in Rats

F344 rats were given a single oral administration of PROJECT P at 300, 1000 and 2000 mg/kg (Study Project P-TX-0001). No deaths occurred. Thus the lethal dose level for male and female rats was higher than 2000 mg/kg. Mucous stool was observed in females in the 1000 mg/kg group. At 2000 mg/kg mucous stool was observed in males and females.

#### Single-dose Oral Toxicity in Dogs

PROJECT P was given as a single oral administration to beagle dogs at a dose of 1000 or

2000 mg/kg (Study Project P-TX-0002). No deaths occurred. Thus the lethal dose level for male and female dogs was higher than 2000 mg/kg. Vomitus was observed in males and females treated with 1000 mg/kg or more. In the females treated with 1000 mg/kg or more, abnormal stool color and decreased body weight, accompanied by decreased food consumption was observed on the day after dosing. In the males dosed with 2000 mg/kg, soft stool and abnormal stool color was observed.

#### Repeated-dose Toxicity

Tabulated results of repeated-dose toxicity studies can be found in End-of-Text Table 3.5 and 3.6.

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

PROJECT P was administered orally once daily for 4 weeks at dose levels of 30, 100, 300, and 1000 mg/kg to male and female F344 rats (Study Project P-TX-0003). At 1000 mg/kg, low hemoglobin concentration was noted in males, and high platelet count and high liver and adrenal weights were noted in females.

In toxicokinetics, Cmax and AUC24 were markedly lower on Day 28 than on Day 1 in males at 300 and 1000 mg/kg and in females at 100, 300 and 1000 mg/kg. However, the overall systemic exposure of PROJECT P increased over the dosing range during the dosing period.

Cmax and AUC24 were generally greater in females than males.

From these results it was concluded that the NOAEL of PROJECT P when administered orally to rats for 4 weeks was 300 mg/kg/day for males and females. Changes noted during the dosing period recovered during the 4-week recovery period.

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

PROJECT P was administered orally once daily for 4 weeks at dose levels of 20, 100, and 1000 mg/kg to male and female beagle dogs (Study Project P-TX-0004).

In the 100 mg/kg group, vacuolation of the epithelium in the gallbladder was observed in males.

In the 1000 mg/kg group, vomiting, salivation and abnormal stool color was observed in all males and females. A high platelet count in both sexes, and high leukocyte count, monocyte count, and neutrophil count and ratio, and a low lymphocyte ratio in 1 male were noted on Day 27 of dosing. High total bilirubin and low total protein, albumin, globulin, and total cholesterol were noted in both sexes on Day 27 of dosing. High blood urea nitrogen, inorganic phosphorus, and sodium, and low calcium in both sexes, and high chloride in males were noted on Day 27 of dosing. In urinalysis, low sodium excretion in males and low chloride excretion in females were noted on Day 26 of dosing. Vacuolation of the epithelium in the gallbladder was observed in both sexes, and vacuolation of the hepatocytes in 1 male, and vacuolation of the epithelium in the duodenum, jejunum, and ileum in 1 female was observed. The vacuoles observed in the hepatocytes and in the epithelium of the gallbladder and small intestine were considered to contain triglyceride as detected by Oil-Red-O stain.

In toxicokinetics, mean Cmax increased dose dependently at 20 and 100 mg/kg, and increased less than the dose ratio at 100 and 1000 mg/kg. Mean AUC24 increased to a greater extent than the dose ratio at 20 and 100 mg/kg, and increased to a lesser extent than the dose ratio at 100 and 1000 mg/kg. Cmax and AUC24 did not change with repeated dosing at any dose level. Mean Cmax and AUC24 were comparable between males and females in all dose groups and at all sampling days.

From these results it was concluded that the NOAEL of PROJECT P when administered orally to dogs for 4 weeks was 20 mg/kg/day for males and 100 mg/kg/day for females. The changes noted during the dosing period recovered during the 4-week recovery period.

#### Genotoxicity

Tabulated results of genotoxicity studies can be found in End-of-Text Tables 3.7 and 3.8.

#### In Vitro Reverse Mutation

The mutagenicity of PROJECT P was examined in the presence and absence of S9 Mix using *Salmonella typhimurium* TA100, TA98, TA1535, and TA1537, as well as *Escherichia coli* WP2 uvrA (Study Project P-TX-0009). PROJECT P inhibited the growth of TA100 at

5000 mcg/plate without metabolic activation. No inhibition was noted in other strains with and without metabolic activation. At doses of 156 to 5000 mcg/plate, PROJECT P did not increase the mean number of revertant colonies by 2-fold more than in the vehicle control group for any bacterial strain tested, regardless of the presence or absence of metabolic activation. These results indicate that PROJECT P has no mutagenic potential.

#### In Vitro Chromosome Aberration

PROJECT P was evaluated for the potential to induce chromosomal aberrations under 3 treatment conditions, i.e., 6-h treatment in the presence of S9 Mix plus 6- and 24-h

treatments in the absence of S9 Mix, using a Chinese hamster lung fibroblast cell line (Study Project P-TX-0010). In the 6- and 24-h treatment groups without metabolic activation,

chromosomal aberrations were analyzed over a concentration range of 150 to 300 mcg/mL and 80 to 120 mcg/mL, respectively. In the 6-h treatment group with metabolic activation, chromosomal aberrations were analyzed over a concentration range of 150 to 250 mcg/mL. PROJECT P did not increase significantly the number of chromosomally aberrant cells when compared to the vehicle control. The results indicate that PROJECT P has no potential to induce chromosomal aberrations.

#### Carcinogenicity

No data for PROJECT P are available.

#### Reproductive and Developmental Toxicity

Tabulated results of reproductive and developmental studies can be found in End-of-Text Tables 3.10 and 3.12.

#### Effects on Embryo-fetal Development in Rats

PROJECT P was administered orally to pregnant SD rats at dose levels of 200 (100×2), 600 (300×2) and 2000 (1000×2) mg/kg/day twice a day (time interval between the 1st and 2nd doses: approximately 6 hours) from Day 7 to 17 of gestation (Study Project P-TX-0007).

In the 600 mg/kg or above groups, a temporary decrease in food consumption was observed in dams. In the live fetuses, a tendency towards an increase in the incidence of visceral variation (thymic remnant in the neck) and high incidence of skeletal variation (bipartite ossification of thoracic centrum) were noted in all dosage groups. In addition, the numbers of the ossified metatarsi were decreased in the 600 mg/kg or more groups and the number of ossified sternebrae were decreased in the 2000 mg/kg group. No test article related effects on the numbers of corpora lutea, implantations or live fetuses, index of embryo-fetal deaths, sex ratio of live fetuses, fetal body weight or placental weight were noted in any dosage group and there were no abnormalities in the placenta or external appearance of live fetuses in any group.

The NOAEL of PROJECT P is estimated to be 200 mg/kg/day for toxicity in dams and less than 200 mg/kg/day for embryo-fetal development.

#### Effects on Embryo-fetal Development in Rabbits

PROJECT P was administered orally to pregnant rabbits at dose levels of 30, 100 or

300 mg/kg/day for 13 days from Day 6 to 18 of gestation (Study Project P-TX-0008). Decreases in fecal output, body weight and food consumption were observed at 300 mg/kg/day as well as abortions in about half of the dams, although no treatment-related findings were observed in gross pathological examination except retention of fur ball in the stomach of the aborting dams. No treatment-related effects such as embryo-fetal death or change in fetal body weight, placental weight or sex ratio including external-gross abnormalities in the fetuses and placentae were observed under these conditions at any dose employed, except for fetal lethality observed at 300 mg/kg/day together with maternal mortality and abortions. In addition, no significant increases were seen in visceral abnormalities or variations, or skeletal abnormalities or variations.

The NOAEL of PROJECT P was judged to be 100 mg/kg/day for general toxicological effect in dams and embryo-fetal development.

#### Local Tolerance

No data for PROJECT P are available.

#### Other Toxicity Studies

No other toxicity data are available for PROJECT P.

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

It is suggested that activation of LPA signaling in the lower urinary tract can induce urethral obstruction, bladder overactivity, and proliferation of prostate stroma; all of which are closely related to the 3 major elements contributing to the pathology of LUTS/BPH: obstruction, clinical symptoms, and hyperplasia. The effects of the novel low molecular weight molecule PROJECT P, a potent and specific LPA1R antagonist, were investigated in animal models related to the mentioned pathological functions. The data for PROJECT P demonstrate its selectivity as an antagonist for LPA1 receptors with nanomolar activity in vitro and a selectivity ratio of ≥1000-fold over other LPA receptors. PROJECT P showed an inhibitory effect on the human NK1 receptor. The Ki value of APProject P for this receptor was

1.4 mcmol/L. The principle of NK1 antagonism is being explored in several clinical development programs (e.g., aprepitant and befetupitant). In the safety pharmacology studies, PROJECT P revealed no effects on the CNS. No affinity was observed for 54 types of receptors, ion-channels, transporters and enzymes.

The in vivo data after iv dosing of PROJECT P at 0.3 mg/kg (minimal effective iv dose) and higher in rats, indicate that basal urethral pressure reduction in anesthetized rats for PROJECT P is higher than that for tamsulosin and point at a more potent urethral relaxation for PROJECT P. Furthermore, it was shown that in in vivo experiments where LPA was found to induce an increase in urethral pressure (Study Project P-PH-0005), or a shortening of the bladder filling cycle (Study Project P-PH-0009), these effects were reversed by PROJECT P at doses of 0.3 mg/kg, iv and higher. PROJECT P (0.1 to 3 mg/kg iv) did not affect the EMG of the external urethral sphincter. In vitro studies with human prostate stromal cells showed inhibition of

LPA-induced prostate cell growth. This profile of activities suggests that this drug is an interesting candidate for development in LUTS/BPH. For estimation of the human effective dose (HED) the efficacy parameter of choice was the decrease of resting UPP in anaesthetized rats (Study Project P-PH-0006) (without infusion of tonus-increasing agents), since the comparator drug tamsulosin showed efficacy to reduce resting UPP in this model at a dose level that is comparable to the HED. The plasma concentration of PROJECT P to reach a similar efficacy as in the mentioned study was determined by iv infusion of PROJECT P under concomitant registration of UPP (Study Project P-PH-0008) and was 563 ng/mL.

The ADME data indicate that the bioavailability of PROJECT P in rats is approximately 55%.

In the safety pharmacology studies, PROJECT P revealed no effects on the CNS, action potential duration or the respiratory system. PROJECT P did inhibit the hERG potassium current in HEK293 cells at 2.5×10−5 mol/L and induced salivation at 100 mg/kg or higher and vomiting and an increase in heart rate at 1000 mg/kg in dogs.

The nonclinical toxicity package consisting of single and repeated-dose studies in rats and dogs, reproductive studies in rats and rabbits, and in vitro genotoxicity studies, supports further development of PROJECT P in males. The main toxicological targets for PROJECT P are the kidney, liver, gastrointestinal (GI) tract and hematopoietic system.

In the repeated-dose toxicity studies increases in blood urea nitrogen, inorganic phosphorus, sodium and chloride, and decreases in calcium and urinary excretion of sodium and chloride were noted in dogs at 1000 mg/kg. No abnormal findings were observed in the histopathology of the kidneys, suggesting a functional change.

Increased liver weight in rats and triglyceride containing vacuoles in the hepatocytes in

1 male dog was observed at the highest dose tested. Although an increase in total bilirubin, decreases in total protein, albumin, globulin and total cholesterol were noted, no adverse histopathological changes were observed in the liver. The findings on the GI tract are related to vomiting and salivation and epithelial vacuolation in gallbladder and duodenum, jejunum and ileum in dogs. The content of vacuoles was, as well as those in the hepatocytes, considered to be triglycerides based on the results of the Oil-Red-O stain. The mechanism for the formation of the vacuoles is unknown, but because of the reduction of cholesterol it could be related to an altered lipid metabolism.

The hematopoietic findings in rats and dogs, i.e., decreased hemoglobin concentration in rats and an increase in platelet count in rats and dogs were not accompanied by histopathological changes in bone marrow, spleen or thymus.

This dataset supports the conduct of a first-in-human (FIH) study in male subjects for up to 4 weeks treatment.

An overview of systemic exposure data of PROJECT P at NOAEL and LOAEL can be found in [[Table 2](#_bookmark61)].

#### Table 2 Compilation of Systemic Exposure Data of PROJECT P at NOAEL and LOAEL

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study No. / Species, Study Duration** | **Dose (mg/kg)** | **Sex** | **Cmax (ng/mL)** | | **AUC24 (ng·h/mL)** | | **Remarks** |
| **First Dose** | **Last Dose** | **First Dose** | **Last Dose** |
| **Project P-PH-0008**  **Rat, single, iv** | 1.2mg/3mL/kg/h | F | Mean Cplasma after 19 min:  563 | NA | NA | NA | Pharmacologically active plasma  concentration |
| **Project P-PT-0004**  **Dog, single, po** | 100 (NOAEL) | M | 116000 | NA | 798000 | NA | NOAEL based on increased heart rate |
| 1000 (LOAEL) | M | 300000 | NA | 2920000 | NA |
| **Project P-TX-0003**  **Rat,4-week, po** | 300 (NOAEL) | M | 54900 | 15200 | 306000 | 41100 | NOAEL based on decreased Hb, and high platelet count and increased liver weight |
| F | 67700 | 45700 | 393000 | 102000 |
| 1000 (LOAEL) | M | 171000 | 36800 | 1870000 | 272000 |
| F | 143000 | 61300 | 1190000 | 224000 |
| **Project P-TX-0004**  **Dog, 4-week, po** | 20 (NOAEL) | M | 41600 | 42900 | 99800 | 97100 | NOAEL based on epithelial vacuolation in gall bladder (M) and vomiting, high platelet count and epithelial vacuolation in gall bladder,  duodenum, jejunum and ileum (F) |
| 100 (NOAEL) | F | 154000 | 136000 | 963000 | 740000 |
| 100 (LOAEL) | M | 178000 | 155000 | 910000 | 956000 |
| 1000 (LOAEL) | F | 285000 | 272000 | 2290000 | 2400000 |

M: Male; F: Female; NA: Not applicable; iv: Intravenous; po: Per os; NOAEL: No observed adverse effect level; LOAEL: Lowest observed adverse effect level; Hb: Hemoglobin.