* + 1. Safety Pharmacology

A total of 4 safety pharmacology studies were performed in accordance with GLP and guidelines of the ICH. These studies are summarized in [[Table 6](#_bookmark34)].

Table 6 Safety Pharmacology Studies with PROJECT G

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Type of study** | **Test System** | **Species, Strain, Sex, Dosing Particulars** | **End Point(s) Measured** | **Major Findings** | **Study No.** |
| hERG potassium current  (in vitro) | Whole-cell patch-clamp technique | hERG channel transfected HEK293 cells, n = 5  Doses: 0.3, 3, 30 µmol/L (up to 16.6 μg/mL) | Inhibition of hERG current | hERG current inhibition 0 µmol/L: 0%  0.3 µmol/L: -4.9%  3 µmol/L: -1.7%  30 µmol/L: -2.3%  (No significant difference with vehicle-control) | Project G-PT-  0004 (GLP) |
| Action potential duration  (in vitro) | Glass electrode technique under a surface- superfusing  condition | Guinea pig, Hartley, male, n = 5,  isolated papillary muscle, Doses: 0.3, 3, 30 μmol/L (up to 16.6 μg/mL) | Resting membrane potential, action potential amplitude, dV/dt max, action potential duration (APD30 and APD90) | No effects on any parameters | Project G-PT-  0001 (GLP) |
| Central nervous system  (in vivo) | Modified Irwin’s  method | Rat, SD, male, n = 6, Doses: 0, 3, 30,  300 mg/kg, po | General activity and behavior | No effects | Project G-PT-  0002 (GLP) |
| Cardiovascular and respiratory systems  (in vivo) | Conscious condition (telemetry system) | Beagle dog, male, n=4, 10, 100,  1000 mg/kg, po | Clinical signs, blood pressure, heart rate, ECG, respiration rate, blood gases, electrolytes, body  temperature | No effects  Other observations: at 1000 mg/kg 1 animal  vomited at 43 h 27 min after dosing | Project G-PT-  0003 (GLP) |

APD30 and APD90: action potential duration at 30% and 90% repolarization; dV/dt max: part of the

action-potential upstroke with the greatest slope; ECG: electrocardiogram; GLP: Good Laboratory Practice; HEK 293: human embryonic kidney 293; hERG: human ether-à-go-go-related gene; SD: Sprague Dawley Source: Studies: Project G-PT-0001, Project G-PT-0002, Project G-PT-0003 and Project G-PT-0004

* + - 1. In-vitro Effects on hERG Current

The effects of PROJECT G on the hERG current were studied in hERG-transfected HEK293 cells by the whole-cell patch-clamp technique in Study Project G-PT-0004. PROJECT G did not affect the hERG current at concentrations of up to 30 μmol/L (approximately 16.6 μg/mL).

* + - 1. Effects on Action Potential Duration in Isolated Papillary Muscles

PROJECT G did not affect the action potential at concentrations of up to 30 μmol/L (approximately 16.6 μg/mL) in isolated guinea-pig papillary muscles using the

glass-electrode technique under a surface-superfusing condition in Study Project G-PT-0001.

* + - 1. In-vivo Effects on Central Nervous System

Rodent general behavior and neurobehavioral function were evaluated by a modified functional observational battery method after single oral administration of PROJECT G to rats at 3, 30 and 300 mg/kg in Study Project G-PT-0002. PROJECT G did not affect the general activity and behavior in any of the rats up to 24 h after administration. The results demonstrate that PROJECT G has no effect on general behavior and the neurobehavioral function at doses of up to 300 mg/kg.

* + - 1. In-vivo Effects on Cardiovascular and Respiratory Systems

PROJECT G was orally administered once to 4 dogs implanted with transmitters of a telemetry system at dose levels of 10, 100 and 1000 mg/kg under conscious conditions in

Study Project G-PT-0003. No test-substance related change was induced at doses up to

100 mg/kg. No effect on body temperature, blood pressure, heart rate, electrocardiogram, respiration rate, blood gasses or blood electrolyte concentrations was noted at doses of up to 1000 mg/kg. At 1000 mg/kg, the following findings were noted: vomiting in 1 animal at 43 h and 27 min after PROJECT G administration and grayish stool in all animals at 8 or 24 h after administration. Vomiting was considered to be incidental due to the time of onset. Grayish stool was considered to have no toxicological significance because it could have been attributed to the unabsorbed test article.

* + - 1. Safety Pharmacology Summary

In summary, no significant effects were observed at or up to the highest PROJECT G concentrations tested (30 μmol/L), in the hERG and action potential duration studies. No notable changes were found up to 300 and 1000 mg/kg in studies to evaluate effects on the CNS and cardiovascular/respiratory systems in rats and dogs, respectively.

### Toxicology

A tabulated overview of nonclinical toxicology studies can be found in [End-of-Text Table 3.1].

* + 1. Single-dose Toxicity

Two single oral toxicity studies were conducted in rats and dogs. The lethal dose was estimated to be higher than 2000 mg/kg for both species.

* + - 1. Single-dose Oral Toxicity in Rats

PROJECT G was orally administered once at dose levels of 1000 and 2000 mg/kg (maximum feasible dose [MFD]) to rats (Study Project G-TX-0001). No animal died and no abnormalities were observed in either group in clinical signs, body weight, food consumption or gross pathology up to 2000 mg/kg.

* + - 1. Single-dose Oral Toxicity in Dogs

PROJECT G was orally administered once at dose levels of 1000 and 2000 mg/kg (MFD) to 1 male and 1 female dog in Study Project G-TX-0002. No animal died or was euthanized in moribund condition up to 2000 mg/kg. Abnormal stool (yellowish white or white or soft

stool) was observed at 1000 mg/kg or more. At 2000 mg/kg, high neutrophil count was noted.

The stool changes were considered to not have toxicological significance since it was possible they were due to unabsorbed test article. No test article-related effects were noted in body weight, food consumption or blood chemistry in either group.

* + 1. Repeat-dose Toxicity

Two 4-week oral toxicity studies in rats and dogs, and 1 additional 4-week toxicity study in rats were conducted to identify the NOAEL. Tabulated results of these studies can be found in [End-of-Text Tables 3.2.1, 3.2.2 and 3.2.3].

* + - 1. 4-week Repeat-dose Oral Toxicity in Rats

PROJECT G was orally administered once daily for 4 weeks at dose levels of 0, 0.3, 3, 30 and 300 mg/kg per day to rats in Study Project G-TX-0003.

No animal died at any dose level, and no toxic changes were noted up to 30 mg/kg per day.

At 300 mg/kg per day, black focus in the mucosa of the glandular stomach with histopathological erosion, vacuolar change of the pancreatic acinar cells and transient soft stool were observed, suppression on body weight gain, decreased serum glucose and a decrease in stool volume (transient) were observed in males and increased serum amylase was observed in females.

In addition to the findings stated above, the following pancreatic findings were observed at

0.3 mg/kg or more: increased pancreas weights at 0.3 mg/kg or more, enlargement of the pancreas at 3 mg/kg or more and hypertrophy of the pancreatic acinar cells at 0.3 mg/kg or more. The pancreatic findings are well known reactions mediated through negative feedback regulation by trypsin of pancreatic exocrine function, to which the rat is especially sensitive; and as such, these findings had little toxicological significance (see [Section [4.4](#_bookmark82)]).

Decreased serum total protein (mainly decreased globulin with increased albumin/globulin (A/G) ratio in males and decreased albumin in females) and decreased total UUN excretion in males were observed at 300 mg/kg per day, and these are probably caused by excessive inhibition of protein absorption; therefore, they were considered to have little toxicological significance. During the 4-week recovery period, the test article-related changes observed during the dosing period recovered or tended to recover (pancreas weight).

In toxicokinetics, Cmax and AUC24 of PROJECT G and its metabolites M1 and M2 increased almost dose proportionally up to 300 mg/kg per day and were similar on days 1 and 28. There was no sex difference in toxicokinetic parameters during the dosing period.

It was concluded that the NOAEL was 30 mg/kg per day for rats. The test article-related changes observed during the dosing period recovered or tended to recover during the 4-week recovery period. The no-observed-effect level (NOEL) on pancreas was not identified in this study.

* + - 1. Additional 4-week Repeat-dose Oral Toxicity in Rats

To investigate the NOEL on the pancreas in rat, an additional 4-week toxicity study was conducted at dose levels of 0, 0.01, 0.03 and 0.1 mg/kg per day (Study Project G-TX-0013) and the analyses were limited to pancreas related parameters.

No test article-related changes were noted up to 0.03 mg/kg per day. At 0.1 mg/kg per day, increased pancreas weight was noted, but no corresponding histopathological findings were observed. It was concluded that the NOEL was 0.03 mg/kg per day.

* + - 1. 4-week Repeat-dose Oral Toxicity in Dogs

PROJECT G was orally administered once daily for 4 weeks at dose levels of 0, 10, 100 and 1000 mg/kg per day to dogs in Study Project G-TX-0004.

No animal died at any dose level, and no relevant adverse changes were noted up to 100 mg/kg per day.

At 1000 mg/kg per day, decreased body weight (approximately -7% to -21% compared to pre-study weight), decreased specific gravity or tendency toward decreased specific gravity in urinalysis, increased serum alkaline phosphatase and amylase, decreased serum calcium, clear cell change of the hepatocytes, acinar cell atrophy in the pancreas and atrophy of the thymus were observed, prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT), increased serum alanine aminotransferase and lipase, dark discoloration of the pancreas, decreased thymus weights and fatty bone marrow were observed in males. In the liver, an increase of glycogen granules in the clear cell changed hepatocytes was observed in electron microscopy. The changes were recovered at the end of the 4-week recovery period.

The following observations reflect the intended pharmacological effect: reduced concentrations of BCAA mainly 1 to 8 h after dosing at 10 mg/kg per day or more, reduced serum urea nitrogen in males at 100 mg/kg per day and reduced serum urea nitrogen, UUN excretion, total protein, albumin and A/G ratio at 1000 mg/kg per day. Since the changes were considered to be related to the excessive inhibition of protein absorption, they were not considered to be toxicologically significant. At 100 mg/kg and more, abnormal stool color (yellowish-white or white) was observed, which was possibly related to the excretion of unabsorbed text article.

In toxicokinetics, Cmax and AUC24 of PROJECT G and its metabolites M1 and M2 increased less than dose proportionally and were at similar at day 1 as at day 28. There were no sex differences in toxicokinetic parameters during the dosing period.

It was concluded that the NOAEL was 100 mg/kg per day for dogs. The test article-related changes observed after the dosing period recovered during the 4-week recovery period.

* + 1. Genotoxicity

Two in vitro genotoxicity studies were conducted.

* + - 1. In-vitro Reverse Mutation

A reverse mutation test was performed with *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2*uvr*A) using the preincubation method with and without metabolic activation in Study Project G-TX-0005. Growth inhibition was not observed at concentrations up to 5000 μg/plate in any test stain with or without metabolic activation. 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 G has no potential to induce gene mutation in bacteria under the conditions of this study.

* + - 1. In-vitro Chromosome Aberration

A chromosome aberration test was performed with cultured mammalian (Chinese hamster lung [CHL]/IU) cells in short-term treatments for 6 h with and without metabolic activation and continuous treatment for 24 h without metabolic activation in Study Project G-TX-0006. The highest dose for chromosomal analysis was set based on the cell proliferation ratio, and

3 lower doses were set for chromosomal analysis. Chromosomal aberrations were analyzed at the following doses: 122, 195, 313 and 500 μg/mL for all treatment conditions. PROJECT G did not induce a statistically significant increase in the number of chromosomal aberrant cells in any treatment group compared with the negative control. It was concluded that PROJECT G has no potential to induce chromosomal aberrations in CHL/IU cells, regardless of the presence or absence of metabolic activation or treatment length.

* + 1. Carcinogenicity

No carcinogenicity studies have been conducted with PROJECT G.

* + 1. Reproductive and Developmental Toxicity

One study each of effects on embryo-fetal development was conducted in rats and rabbits. Neither teratogenicity nor lethal effects were observed in the fetuses of either rats or rabbits.

* + - 1. Effects on Embryo-fetal Development in Rats

PROJECT G was orally administered from day 7 to day 17 of gestation at dose levels of 100, 300 and 1000 mg/kg per day to pregnant rats (Study Project G-TX-0010). The exposures in the pregnant rats are summarized in [[Table 7](#_bookmark77)]. In dams, suppression of body weight gain was noted during the dosing period at 100 mg/kg per day or more. Decreased food consumption was noted in the early phase of the dosing period at 1000 mg/kg per day. In fetuses, decreased fetal body weight and placental weight were noted at 1000 mg/kg per day. The observed effects are probably related to the reduction of protein intake during the study, which is the pharmacological effect of PROJECT G. It was concluded that the NOAEL was less than 100 mg/kg per day for dams and 300 mg/kg per day for embryo-fetal development.

Table 7 Exposures of PROJECT G after the Final Administrations (Total and Unbound)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Number/Sex/Species** | **Dose (mg/kg)** | **Unchanged Drug** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng·h/mL)** | |
| **Total** | **Unbound**† | **Total** | **Unbound**† |
| 3 pregnant rats | 100 | 1142.12 | 0.65 | 16910.57 | 9.59 |
| 3 pregnant rats | 300‡ | 2323.00 | 1.32 | 36920.93 | 20.93 |

† The unbound concentration has been calculated based on the plasma protein binding ratio; the unbound fraction used in the calculations was 0.000567 (Study Project G-ME-0010).

‡ Fetal no-adverse-effect level.

Source: Studies Project G-ME-0010 and Project G-TX-0010

* + - 1. Effects on Embryo-fetal Development in Rabbits

PROJECT G was orally administered from day 6 to day 18 of gestation to pregnant rabbits at dose levels of 30, 100 and 300 mg/kg per day (Study Project G-TX-0012). The exposures in the pregnant rabbits are summarized in [[Table 8](#_bookmark79)]. In dams, significantly decreased body weight and/or body weight gain were noted during the dosing period at 100 mg/kg per day or more. A decrease in stool volume was noted at 300 mg/kg per day. In the 100 mg/kg per day group, 1 of the 19 dams aborted her litter on day 22 of gestation. No test article-related changes

were noted in the number of live fetuses, number of embryo-fetal deaths, postimplantation loss rate, fetal body weight, placental weight, sex ratio or external, placental, skeletal or visceral findings up to 300 mg/kg per day. It was concluded that the NOAEL was 30 mg/kg per day for dams and 300 mg/kg per day for embryo-fetal development.

Table 8 Exposures of PROJECT G after the Final Administrations (Total and Unbound) (Study Project G-TX-0012)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Number/Sex/Species** | **Dose (mg/kg)** | **Unchanged Drug** | | | |
| **Cmax (ng/mL)** | | **AUC24 (ng·h/mL)** | |
| **Total** | **Unbound**† | **Total** | **Unbound**† |
| 3 pregnant rabbits | 30‡ | 1150.27 | 0.96 | 17541.15 | 14.6 |
| 3 pregnant rabbits | 300§ | 17462.71 | 14.5 | 287949.24 | 240 |

† The unbound concentration has been calculated based on the plasma protein binding ratio; the unbound fraction used in the calculations was 0.000833 (Study Project G-ME-0010).

‡ Maternal no-adverse-effect level.

§ Fetal no-adverse-effect level.

Source: Studies Project G-ME-0010 and Project G-TX-0012

* + 1. Local Tolerance

No local tolerance toxicity studies have been conducted with PROJECT G.

* + 1. Other Toxicity Studies

An in vitro 3T3-NRU phototoxicity study was performed with cultured mammalian cells (Balb/c 3T3 cells) at 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 μg/mL in the presence and absence of UV-A irradiation (Study Project G-TX-0007). The mean photo effect (actual value: −0.014) was less than 0.1. Therefore, PROJECT G was categorized as having no potential for phototoxicity.

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

The nonclinical safety profile of PROJECT G has been evaluated according to ICH M3 guidelines and all findings were evaluated for relevance to human risk.

PROJECT G is an inhibitor of trypsin with Ki values of 13.1 and 17.9 nmol/L against human and rat trypsin, respectively. Screening for off-target pharmacological activity identified leukotriene B4 with a Ki value of 1.42 μmol/L, and human Factor Xa, Factor VIIa and kallikrein as potential targets.

The effect of PROJECT G on the trypsin-mediated increase in rat plasma BCAA in response to skimmed milk intake was determined in normal rats and in 5/6 Nx CKD rats. PROJECT G significantly inhibited the increase of plasma BCAA following skimmed milk administration in normal and 5/6 Nx rats at 3 mg/kg.

In terms of beneficial clinical effects, PROJECT G has demonstrated reduced progression of renal insufficiency and kidney injury/fibrosis in models of both early stage DKD (KK/Ay diabetic mice) and late stage CKD (5/6 Nx rats), respectively. In 5/6 Nx rats, PROJECT G significantly improved proteinuria and renal function (Ccr) at 3 mg/kg bid. In addition, 5/6 Nx rat UUN excretion was significantly reduced with PROJECT G treatment. When

PROJECT G was administered in combination with losartan (angiotensin II receptor blocker) or enalapril (angiotensin converting enzyme inhibitor), additive renoprotective effects on proteinuria, renal function and histopathological changes compared with losartan or enalapril alone were achieved. PROJECT G was effective when administered alone or in combination with losartan or enalapril in the KK/Ay mouse model of DKD.

In the human setting, it is well known that during CKD/DKD progression, protein absorption contributes to the decline in renal mass and function, as exemplified by the beneficial influence of adhering to a strict LPD. In the mouse model of DKD (KK/Ay mice), 3 mg/kg PROJECT G twice daily had additive effects when combined with either enalapril

(angiotensin-converting enzyme inhibitor, 3 mg/kg once daily) or losartan (angiotensin receptor blocker, 10 mg/kg once daily) on UACR and UUN in comparison to enalapril or losartan alone. Similarly in the 5/6 Nx rat CKD model, 3 mg/kg PROJECT G twice daily had additive effects when combined with either enalapril (3 mg/kg once daily) or losartan

(10 mg/kg once daily) on UPCR, blood urea nitrogen and Ccr.

Collectively, the nonclinical pharmacology data set described above implies that PROJECT G may slow the rate of progression and delay time to dialysis in CKD and DKD patients when administered as an add-on to the current standard therapies like angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

No indication of adverse effects were observed at or up to the highest concentrations tested of PROJECT G (30 μmol/L), in the hERG and action potential duration studies or up to 300 or 1000 mg/kg in studies to evaluate effects on the CNS and cardiovascular/respiratory systems in rats and beagle dogs, respectively.

Single- and repeat-dose toxicity studies have been performed in rats and dogs with a once daily dose of PROJECT G up to a duration of 4 weeks with a 4-week recovery period. The NOAEL was 30 mg/kg in rats and 100 mg/kg in dogs. The adverse reaction profile of PROJECT G was primarily related to its pharmacological activity. At high doses, the rats and dogs showed protein malnutrition induced effects, like reduction in body weight. Toxicity studies that have been performed showed a high lethal dose (> 2000 mg/kg) in rats and dogs. The main targets for PROJECT G in the repeat-dose toxicity studies were the pancreas, GI tract, liver and hematopoietic system at the highest doses only, 300 mg/kg per day in rats and 1000 mg/kg per day in dogs.

The response of the pancreas to treatment showed differences between rats and dogs. The effects observed in rats are similar to the known effects induced by trypsin inhibitors (e.g., soy protein) in rodents [Göke, 1990]. Treatment of rats with trypsin inhibitors (raw

soya flour, camostat and also CCK agonists) is known to cause a similar trophic response in the acinar part of the pancreas due to increased circulating plasma levels of CCK [Myer et al, 2014, Greaves, 2012, Göke et al, 1986]. This response has been observed in rat, mouse and chicken, but not in calf, dog or marmoset monkey [Göke, 1990]. The mechanism of pancreatic effects in the rat is well understood to be due to an interruption of the feedback mechanism of pancreatic exocrine function [Greaves, 2012]. The rat is exquisitely sensitive to interrupted exocrine pancreatic feedback, which has been shown to result in induction of pancreatic acinar carcinoma (after long-term administration of raw soya flour); this is potentially the result of a high CCK1 receptor density found on rat acinar cells, which are functionally absent in humans [Pandiri, 2014, Wang & Cui, 2007]. Whilst hypertrophic

responses of the pancreas to CCK increase are observed in several species including humans [Friess et al, 1998], the rat is particularly sensitive and is the only species where a transition into carcinomas after long-term increase (by soya flour) has been observed [McGuinness et al, 1987]. If a CCK feedback mechanism is present in humans, the consequence of increased CCK will differ from rodents since the cholinergic system is more active in the regulation of trypsin in humans than in rats [Owyang, 1994]. Therefore, the rat pancreas is more sensitive to increased CCK stimulation of acinar cells. Acinar cell atrophy in the dog pancreas is a more generally observed phenomenon and is most probably related to excessive inhibition of protein intake since it is also observed in patients with protein deficient malnutrition [Brooks & Golden, 1992]. Thus, the consequence of pancreatic hyperplasia in terms of acinar carcinogenicity is not recognized as a human response to protease inhibition. Indeed, there is no recognized association between human intake of soya (trypsin inhibitor that causes pancreas neoplasms in rats) and pancreatic cancer [Michaud, 2004].

In repeat-dose dog studies, principal findings were related to excessive pharmacology and reduced protein intake such as reduced plasma BCAA (10 mg/kg), reduced serum urea nitrogen, UUN and abnormal stool color (100 mg/kg) and body weight reduction by 7% to 21% (1000 mg/kg). Acinar cell atrophy in the pancreas, atrophy in the thymus and fatty bone marrow in dogs observed at 1000 mg/kg are linked to protein deficiency and were reversible after 4 weeks recovery. Thus, the NOAEL in dogs was set at 100 mg/kg based on body weight loss.

Findings at the lowest-observed-adverse-effect-level (LOAEL) in the toxicity studies were all reversible and considered feasible to be monitored in clinical studies. Furthermore, it is expected that prevention of excessive pharmacological response will prevent most of the observations at the LOAEL.

PROJECT G revealed no in vitro genotoxic potential, no teratogenic potential in rats or rabbits and no in vitro phototoxic potential.

4.4.1 Exposure Assessment

Data from the skimmed milk protein loading study in normal rats (Study Project G-PH-0006) were considered most relevant to determine the maximum recommended starting dose. In this study, the inhibition of protein absorption was examined in rats that were administered PROJECT G 0.3, 1, 3 and 10 mg/kg orally immediately prior to an oral skimmed milk loading. Statistically significant inhibitions were observed at doses of 3 and 10 mg/kg. Based on these results and the rat body surface area-conversion factor of 0.16, the minimal pharmacological active dose (PAD) is judged to be 3 mg/kg in rats, and the human equivalent dose of this PAD is 28.8 mg per subject (3 mg/kg x 0.16 x 60 kg). After applying a default factor of 10, which allows for variability in extrapolating animal pharmacology to human studies, the maximum recommended starting dose based on pharmacology is determined to be 2.88 mg per subject. This is about 10-fold lower than the maximum recommended starting dose based on toxicology.

The nonclinical safety package does not indicate adverse effects that necessitate setting a maximum plasma concentration/exposure. The hypertrophy and vacuolization of the pancreatic acinar cells found in rats is established as a rat-specific response to trypsin inhibition and this is not relevant to the human situation. Other effects that are the result of excessive pharmacology and protein restriction, reduced systemic protein and glucose were reversible, can be easily monitored in humans and are typically non-life threatening; and adequate treatment options are available. For the start of the first-in-human study, exposure limits were calculated.

The highest exposure was observed in dogs at the top dose of 1000 mg/kg. This is the LOAEL in the study and the findings indicate signs and symptoms of protein energy malnutrition (7% - 21% bodyweight loss). Since this is based on exaggerated pharmacology of trypsin inhibition (i.e., reduced protein absorption), which will be monitored in clinical studies, it can be justified to set the exposure limit (AUC0-24) and Cmax at this dose level.

The highest individual plasma exposure (AUC0-24) observed on the last day of the top dose in the 4-week repeat-dose study in male dogs is 11856.89 ng·h/mL, which corresponds to an unbound AUC0-24 of 1743 ng·h/mL (mean unbound fraction of 14.7% in dogs). Taking into account a human free fraction of 1.25%, the corresponding human total exposure would be 139440 ng·h/mL or approximately 139 µg·h/mL.

The highest individual plasma concentration (Cmax) observed on the last day of the top dose in the 4-week repeat-dose study in male dogs is 2300.61 ng/mL, which corresponds to an unbound maximum Cmax of 338.2 ng/mL (free faction of 14.7% in dogs). Taking into account a human free fraction of 1.25%, the corresponding human maximum Cmax would be 27056 ng/mL or approximately 27 µg/mL.

The exposure limit is set at 139 µg·h/mL and the limit for Cmax is set at 27 µg/mL based upon the highest exposure observed in dogs (male dogs 1000 mg/kg, LOAEL).

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