### Safety Pharmacology

A total of 4 safety pharmacology studies were performed in accordance with Good Laboratory Practice and guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Tabulated results of safety pharmacology studies can be found in [End-of-Text Table 1.2].

### Effects on CNS

PROJECT H was orally administered as single dose to male Sprague Dawley rats at dose levels of 1, 10, 30 and 60 mg/kg [Project H-PT-0003]. Rodent general behavior and neurobehavioral function were evaluated by a modified functional observational battery.

At 1 mg/kg, no effects were observed. At 10 mg/kg or more, a decrease in locomotor activity, reddening of skin and a dose-dependent decrease in rectal temperature were noted. At

30 mg/kg or more, incomplete eyelid opening, slight flaccidity in abdominal tone, prone position, a low level of arousal, soft stool, limp extensor thrust reflex were noted. In addition, bradypnea, a decrease in the escape response, disappearance of proprioceptive positioning reaction, piloerection were also noted at 60 mg/kg PROJECT H. All findings disappeared within 24 h.

### Effects on hERG Current

The effects of PROJECT H on the hERG current were studied in hERG-transfected HEK293 cells by the whole-cell patch-clamp technique [Project H-PT-0001]. PROJECT H suppressed the hERG current in hERG-transfected HEK293 cells in a concentration-dependent manner with an IC50 of 3.6 μmol/L (approximately 1.1 μg/mL).

### Effects on Action Potential Duration in Isolated Papillary Muscles

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

[Project H-PT-0002]. PROJECT H did not affect the action-potentials at concentrations of up to

2.69 μmol/L (0.808 μg/mL). At 9.23 μmol/L (2.77 μg/mL), PROJECT H shortened APD30 (−6.1%), prolonged APD30-90 (20.6%), and decreased dV/dt max (−30.1%).

### 4.1.3.4 Effects on Cardiovascular and Respiratory Systems

PROJECT H was orally administered once to 4 cynomolgus monkeys implanted with transmitters of a telemetry system at dose levels of 1, 3, 10, 30 and 60 mg/kg under unanesthetized conditions [Project H-PT-0004]. There were no changes at 1 mg/kg. At 10 mg/kg or more, decreases in DBP, MBP, and heart rate, prolongation of PR interval, increase in arterial carbon dioxide tension were noted. In addition, a suppression of diurnal elevation of intra-abdominal body temperature and transient decrease in the body temperature were observed at 10 and 30 mg/kg or more, respectively. At 30 mg/kg or more, vomiting, crouching position, and a decrease in food consumption were noted.

## Toxicology

A total of 10 definitive GLP toxicity studies and 3 preliminary studies (including in vitro studies) were conducted in rats, cynomolgus monkeys, and rabbits. All definitive studies were performed in accordance with Good Laboratory Practices and guidelines of International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. An overview of toxicology studies of PROJECT H can be found in [End of-Text Table 3.1].

### Single-dose Toxicity

Two definitive single-dose toxicity studies were conducted.

### Single-dose Oral Toxicity in Rats

PROJECT H was orally administered once at dose levels of 60, 200, and 300 mg/kg to male rats and 30, 100, and 300 mg/kg to female rats, respectively [Project H-TX-0001]. All males and

4 out of 5 females died within 6 h after dosing at 300 mg/kg. At 300 mg/kg, clonic convulsion, lateral position, gasping, salivation and moist fur around urethral orifice were observed. Skin flushes were observed at 30 mg/kg or more, and decreased spontaneous motility and salivation were noted at 60 mg/kg or more. A slight and transient decrease in body weight was noted at 200 mg/kg.

### Single-dose Oral Toxicity in Cynomolgus Monkeys

PROJECT H was orally administered once at dose levels of 30 and 60 mg/kg to 1 male and

1 female cynomolgus monkeys [Project H-TX-0002]. No animal died or was euthanized due to moribundity at any dose. A decrease or tendency of decrease in food consumption was noted in both animals on day of dosing at 30 mg/kg or more. Vomiting and salivation were observed in both animals from 0.5 to 5 h after dosing at 60 mg/kg, in addition, retching was observed in the female at 0.5 h after dosing.

### Repeat-dose Toxicity

One preliminary 1-week oral toxicity study in cynomolgus monkeys and two definitive 4-week oral toxicity studies in rats and cynomolgus monkeys, and one additional 4-week toxicity study in rats were conducted. Tabulated results of these studies are provided [End-of-Text Tables 3.5, 3.5.1, 3.5.2.and 3.5.3].

### Definitive 4-Week Repeat-dose Oral Toxicity in Rats

PROJECT H was orally administered to rats once daily for 4 weeks at dose levels of 0, 1, 10, 30, and 60 mg/kg per day to males and 0, 1, 3, 10, and 30 mg/kg per day to females.

[Project H-TX-0003].

There were no PROJECT H-related changes in males at 1 mg/kg and females up to 3 mg/kg. At 10 mg/kg per day or more, skin flushes were observed in both sexes. Decrease in urinary specific gravity or increase in urinary volume were noted in males at 10 mg/kg per day or more and females at 30 mg/kg per day. A decrease in food consumption, and centrilobular hypertrophy in the liver were observed in both sexes at 30 mg/kg per day and males at

60 mg/kg per day, in addition, suppressed body weight gain, shortening of prothrombin time (PT) and activated partial thromboplastin time (APTT) were noted in males. Salivation, increases in total cholesterol, alanine aminotransferase (ALT), relative liver weight, hypertrophy of follicular cells in the thyroid, and erosion in the glandular stomach were observed in females and males at 30 and 60 mg/kg per day, respectively. Suppressed body weight gain, decreases in body temperature, serum triglyceride and glucose, the weights of the spleen and thymus, increases in serum total cholesterol, inorganic phosphorus calcium, the weights of the liver and thyroids were noted in males at 60 mg/kg per day. At this dose level, white pulp atrophy in the spleen, thymic atrophy, granulomatous inflammation and bronchiolar epithelial hypertrophy and alveolar foam cell in the lung were noted, in addition, vacuolar changes in the proximal tubules in the kidney, epithelium in the epididymis and the retinal pigment epithelium in the eye which suggests phospholipidosis were observed in one male rat. Vacuolated lymphocyte ratio and serum level of di-22:6-BMP [Project H-TX-0018] were increased at week 4 of dosing.

After a 4-week recovery, the findings except for the decrease in urinary specific gravity and an increase in urine volume were recovered or were partially recovered. The reversibility of vacuolar changes in multiple organs could not be evaluated because of the low incidence. It was concluded that the NOAELs in this study were 1 and 3 mg/kg per day for males and females, respectively.

### Additional 4-Week Repeat-dose Oral Toxicity in Rats

To evaluate the reversibility of the decreased urinary specific gravity and increases in urinary volume and vacuolar changes, an additional 4-week toxicity study with 13-week recovery was conducted using male rats at PROJECT H dose of 30 and 60 mg/kg per day. Twenty and 60 male rats were dosed 30 and 60 mg/kg per day, respectively [Project H-TX-0019]. The systemic exposure levels were similar to the previous study [Project H-TX-0003]. Dose dependent increases in urinary volume and water intake and a decrease in urinary specific gravity were reproduced. The changes were reversible and after a 4 week recovery period levels had returned to normal. The vacuolar changes were not observed in the second study, however, both urine and serum levels of di-22:6-BMP increased dose dependently at week 4 of dosing. The di-22:6-BMP concentrations returned to control levels by 4-week recovery. It was concluded that the urinary changes caused by a 4-week treatment were reversible. No vacuolization or phospholipidosis was observed in any of the 30 animals dosed with

60 mg/kg per day PROJECT H in a second 4-week repeated dose study.

### Exploratory 1-Week Repeat-dose Oral Toxicity in Cynomolgus Monkeys

AS256Project H-FM (fumarate of PROJECT H) was orally administered at dose levels of 3, 10, and 30 mg/kg per day to 1 male and 1 female cynomolgus monkey per group once daily for 1 week [Project H-TX-0015].

There were no changes in animals at 3 mg/kg per day and the female at 10 mg/kg per day, suggesting the NOAEL. A decrease in heart rate was noted in the male at 10 mg/kg per day at 6 h after the first dose. In the female dosed with 30 mg/kg per day, clonic convulsion, sitting or lateral position, and disappearance or suppression of touch response were observed at 1 h after dosing on days 2 and 4. After the convulsion on day 4, vertical nystagmus was observed. Decreased spontaneous activity and vomiting were also observed in the female on days 2 to 4. These observations were transient and were not observed 4 h or later after dosing. The dose level for the female in the highest dose group started at 30 mg/kg and then decreased to 20 mg/kg on day 5 and later since clonic convulsion was observed in the

30 mg/kg dosed female on days 2 and 4. Decreases in body weight, food consumption, body temperature and heart rate, and prolonged corrected QT (QTc) interval were observed in the female. In the male at 30 mg/kg per day, decrease in body temperature and blood pressure, spontaneous activity, food consumption and body weight, suppression of touch response, sitting position, vomiting and salivation were observed. Under the conditions of the present study, the NOAEL of AS256Project H was 3 and 10 mg/kg per day for male and female, respectively.

### 4-Week Repeat-dose Oral Toxicity in Cynomolgus Monkeys

PROJECT H was orally administered to 4 male and 4 female cynomolgus monkeys for 4 weeks at dose levels of 0, 3, 10, and 20 mg/kg per day [Project H-TX-0004].

There were no changes at 3 mg/kg per day. A decrease or tendency toward a decrease in body weight and decreases in food consumption and albumin/globulin ratio, and increased globulin were observed at 10 mg/kg per day or more. At 20 mg/kg per day, decreases in

spontaneous activity (slight) and body temperature, vomiting, and salivation were observed. In addition, the following findings are observed: prolongation of both QRS duration and PR interval in 1 male, , decreases in hemoglobin concentration and hematocrit value and increased reticulocyte ratio in 1 male, decreased albumin in 1 male, increased platelet in 2 males and 2 females and eosinophil and large unstained cell counts in 2 females each. The relative liver weight increased in both sexes at 20 mg/kg per day. Although various findings and changes were observed as mentioned above, no histopathological findings were detected in any organs. All findings observed in the dosing period at 20 mg/kg per day disappeared after a 4-week recovery period.

It was concluded that the NOAEL was 3 mg/kg per day.

### Genotoxicity

Two definitive in vitro genotoxicity studies were conducted.

### In Vitro Reverse Mutation

A reverse mutation test was performed with *Salmonella typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (WP2 uvrA), using the preincubation method with and without metabolic activation [Project H-TX-0005]. PROJECT H inhibited the growth of all the strains at 625 μg/plate without metabolic activation and 1250 μg/plate with metabolic activation. No precipitation on the plate was observed with any treatment. The mean number of revertant colonies did not reach double that of the negative control for any strain at any concentration, regardless of the presence or absence of metabolic activation. It was concluded that PROJECT H has no potential to induce gene mutation in bacteria.

### In Vitro Chromosome Aberration

A chromosome aberration test was performed with cultured mammalian (CHL) cells in

short-term treatments for 6 h with and without metabolic activation, and continuous treatment for 24 h without metabolic activation [Project H-TX-0006]. In the 6 h treatment with and without metabolic activation, chromosomal aberrations were analyzed over a concentration range of 60 to 70 μg/mL and 35 to 45 μg/mL, respectively. In the 24 h treatment group without metabolic activation, chromosomal aberrations were analyzed over a concentration range of

5 to 10 μg/mL.

PROJECT H did not induce statistically significant increase in the number of chromosomal aberrant cells compared with the negative control. It was concluded that PROJECT H has no potential to induce chromosomal aberrations in CHL cells.

### Carcinogenicity

No carcinogenicity studies have been performed with PROJECT H so far.

### Reproductive and Developmental Toxicity

Two definitive embryo-fetal development studies in rats and rabbits with dose-range finding studies were conducted.

### Effects on Embryo-fetal Development

**4.3.5.1.1 Effects on Embryo-fetal Development in Rats**

PROJECT H was administered orally from day 7 to day 17 of gestation at dose levels of 3, 10, and 30 mg/kg per day to pregnant rats [Project H-TX-0009]. In dams, skin flushes (reddening) was observed at 3 mg/kg per day or more. Decreased food consumption was noted at

10 mg/kg per day or more, in addition, low body weight and suppressed body weight gain were noted at 30 mg/kg per day, respectively. In fetuses dosed with 30 mg/kg per day PROJECT H, low body weight and low numbers of ossified sternebrae and sacral and caudal vertebrae suggesting growth retardation, likely due to maternal toxicity, were observed. It was concluded that the NOAEL was less than 3 mg/kg per day for dams and 10 mg/kg per day for embryo-fetal development.

### Effects on Embryo-fetal Development in Rabbits

PROJECT H was orally administered from day 6 to day 18 of gestation to pregnant rabbits at dose levels of 1, 3, and 10 mg/kg per day [Project H-TX-0010]. In dams, suppressed body weight gain and decreased food consumption were noted at 10 mg/kg per day. No test article-related changes were noted in the number of live fetuses, number of embryo-fetal deaths, postimplantation loss rate, sex ratio, or external, placental, skeletal, or visceral findings up to 10 mg/kg per day.

It was concluded that the NOAEL was 3 mg/kg per day for dams and 10 mg/kg per day for embryo-fetal development.

### Local Tolerance

No studies have been performed with PROJECT H so far.

### Other Toxicity Studies

An in vitro 3T3-NRU phototoxicity study was performed with cultured mammalian cells (Balb/c 3T3 cells) at 33.2, 46.5, 65.1, 91.1, 128, 179, 250, and 350 μg/mL in the presence and absence of UV-A irradiation [Project H-TX-0016]. The IC50 was calculated in both the presence and absence of irradiation, and the photo irritation factor (actual value: 1.017) was less than 2. Therefore, PROJECT H was categorized into no phototoxicity.

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

SUI is a symptom defined by the International Continence Society as involuntary leakage from the urethra, synchronous with exertion/effort, or sneezing or coughing [Abrams et al, 2002]. The urinary continence mechanisms include two different pathways, a sympathetic and a somatic storage reflex. When the bladder fills, it accommodates bladder expansion which maintains a low bladder pressure. As filling increases, activation of the spinal sympathetic reflex induces detrusor muscle relaxation, and activation of α-adrenergic receptors in the urethral smooth muscle increases the outlet resistance. The spinal reflex mediated by pelvic nerve afferent fibers also causes activation of efferent pudendal motoneurons in Onuf’s nucleus, which is located in the ventral horn of the sacral spinal cord, resulting in contractile responses of the striated (external) urethral sphincter. The urethral rhabdosphincter activity is partially controlled by serotonergic and adrenergic receptors in the Onuf’s nucleus. Conscious or reflexive valsalva maneuvers such as coughing, sneezing and laughing activate pudendal motoneurons in Onuf’s nucleus. This reflex contraction is thought to be involved in the quick response in maintaining continence during sudden increases in abdominal pressure.

Based on the physiological continence mechanisms, the concept of promoting the motor neuron reflex to close the external urethral sphincter pharmacologically is thought to be an appropriate approach in the treatment of SUI. Duloxetine is the only pharmacological treatment of SUI related to this concept. . This serotonin (5-HT) /norepinephrine reuptake inhibitor, approved in some EU countries, activates 5-HT2 and α1-adrenergic receptors on sacral pudendal motoneurons in the Onuf’s nucleus resulting in increased serotonin and norepinephrine availability and consequent EUS activation [Jost and Marsalek, 2003; Thor and Katofiasc, 1995].

5-HT2C receptors in humans are predominantly distributed in the CNS including the Onuf’s nucleus, but little or none are found in peripheral tissues. The only 5-HT2C selective agonist on the market is lorcaserin, which was approved by the FDA (but not in EU) for the treatment of obesity.

In addition to 5-HT2C, PROJECT H was found to be an agonist of human 5-HT1A, 5-HT2A, and 5-HT7 receptors [Project H-PH-9011] and an antagonist of human 5-HT2B and β2 adrenoceptors. The functional selectivity of PROJECT H for human 5-HT2C over these receptors is >100 fold based on the EC50, or IC50 value.

In the guinea pig cystometry experiment, PROJECT H enhanced EUS-EMG activity at iv doses of 1 and 3 mg/kg and increased intra-urethral pressure during the filling phase at an iv dose of 3 mg/kg. This effect on EUS-EMG activity was blocked by a 5-HT2C receptor antagonist. Duloxetine is reported to increase EUS-EMG activity in anesthetized cats with acetic acid irritated bladders [Thor KB and Katofiasc MA, 1995]. In the same model, PROJECT H showed increase in EUS-EMG activity 200% over vehicle effect at 0.03 mg/kg iv. The corresponding unbound plasma level was calculated to be 0.63 ng/mL, which is 2.7-fold higher than the in vitro EC50 for PROJECT H for cat 5-HT2C receptors. These data suggest that PROJECT H at an unbound plasma level of 0.63 ng/mL might have clinical effectiveness in incontinence episode frequency in SUI patients.

Cmax and AUCinf increased more than dose-proportionally after a single oral administration (1 to 10 mg/kg) in rats and monkeys. The oral bioavailability increased with dose, with 18.1% for rats and 3.6% for monkeys at 1 mg/kg, while at 10 mg/kg, it was 83.4% and 24.2%, respectively. Brain penetration studies showed good CNS transfer of PROJECT H in all animals tested (rats, guinea pigs, and cats).

The in vitro plasma protein binding ratio of PROJECT H was 73.87% to 86.17% in mouse, rat, rabbit, dog, monkey, cat, and human. LDL and HDL were considered to be the major binding proteins of PROJECT H in human plasma. Enterohepatic circulation was found in rats.

No human-specific PROJECT H metabolites were formed in vitro by liver microsomes or hepatocytes. The main isoform responsible for CYP-mediated metabolism of PROJECT H in humans was CYP3A4/5, while CYP1A2 and CYP2D6 may also be involved. Irreversible inhibition of CYP2D6 was observed. PROJECT H is expected to have a low in vivo P-gp liability, and good CNS penetration is expected in humans.

In the in vitro safety pharmacology studies, PROJECT H suppressed the hERG current with an IC50 of 3.6 μmol/L (1.1 μg/mL) and shortened APD30, prolonged APD30-90, and decreased dV/dt max at 9.23 μmol/L (2.77 μg/mL) in isolated guinea-pig papillary muscle. These concentrations are 1750-fold and 4400-fold higher than Ceff,u, and therefore, PROJECT H is unlikely to prolong the QT interval at pharmacologically relevant plasma exposure levels. In the CV safety pharmacology study in cynomolgus monkeys, decreased diastolic blood pressure and heart rate and prolonged PR interval were observed at 10 mg/kg or more

(Cmax,u is 73x Ceff,u) [[Table 2](#_bookmark67)], occurring around the tmax. In the 4-week repeated dose toxicity study in cynomolgus monkeys, prolongation of QRS duration was observed at 20 mg/kg per day or more (Cmax,u was 66-fold higher than Ceff,u). The mechanism of these rate changes has not been determined. Nevertheless, since there was no baroreflex response between blood pressure and heart rate, an indirect action via suppression of CNS rather than a direct action on blood vessels or heart is considered. Flushed skin due to increased cutaneous blood flow was observed in rats only.

CNS effects, such as decreases in general activity, body temperature, food consumption and body weight (gain) were observed in both rats and cynomolgus monkeys. These CNS effects could be either attributed to the primary pharmacological activity of PROJECT H, or to activation of secondary target receptors (5HT1A, 5HT2A and 5HT7). In the preliminary

1-week repeat dose toxicity study, clonic convulsions were observed in rats at the lethal dose (300 mg/kg) after a single oral dose and at a dose of 30 mg/kg per day in one female monkey. No convulsions occurred in the 4-week GLP study (3, 10, 20 mg/kg/day).

In the 4-week toxicity study in rats, vacuolar changes in the kidney, epididymis and eye were observed in 1 male animal at 60 mg/kg per day. The vacuolation was considered suggestive of phospholipidosis. An additional 4-week repeated dose study was conducted, specifically aimed at characterizing the phospholipidosis occurrence and reversibility in a larger group of male rats. However, in this study, no vacuolization or phospholipidosis was observed in any of the 30 animals dosed with 60 mg/kg per day PROJECT H. Urine and serum levels of

di-22:6-BMP increased dose dependently at week 4 of dosing but returned to control levels by 4-week recovery.

In the 4-week repeated dose toxicity study in rats, increased liver and thyroid weights were noted in males at 60 mg/kg per day and females at 30 mg/kg per day; ALT activity also increased in these females. Histopathological examination revealed centrilobular hypertrophy in the liver, with smooth endoplasmic reticulum proliferation confirmed by electron microscopy. There was no evidence of hepatocellular damage. This suggests that the centrilobular hypertrophy in the liver was caused by induction of hepatic drug-metabolizing enzymes as an adaptive change of hepatocytes [Amacher et al, 1998; Popp and Cattley, 1998]. Thyroid follicular cell hypertrophy is a well-known phenomenon that occurs secondary to an increased hepatic thyroid hormone glucuronidation [Wu and Farrelly, 2006]. These findings resolved after 4-weeks of drug cessation.

In monkeys, decreases in erythrocyte count, hemoglobin, and hematocrit and increases in platelets, eosinophil and large unstained cell counts occurred. In rats, white pulp atrophy in the spleen was observed. Standard monitoring of hematological parameters (hemoglobin, hematocrit, RBC counts, and reticulocyte counts) in clinical studies is sufficient to detect any hematological effects of PROJECT H in humans.

PROJECT H revealed no genotoxicity potential in the in vitro reverse mutation and chromosome aberration tests. In addition, PROJECT H did not induce phototoxicity in the in vitro 3T3 NRU assay.

In the embryo-fetal development studies in rats and rabbits, PROJECT H was not teratogenic, but did show maternal toxicity (e.g., low body weight, decreased body weight gain and food consumption). In rats, growth retardation in fetuses was observed at the highest dose level. The NOAELs for dams were less than 3 mg/kg per day in rats and 3 mg/kg per day in rabbits, and the NOAELs for embryo-fetal development were 10 mg/kg per day in both rats and rabbits.

Overall, several toxicological findings were identified in the safety pharmacology and toxicity studies. These included effects on CNS, cardiovascular and respiratory systems, increase in urinary volume, and phospholipidosis-like changes. The safety margins on some of these findings were small or less than one [[Table 2](#_bookmark67)], however, the findings with small safety margins are of mild severity, are reversible and can be monitored in phase 1 clinical studies. The relevance of the safety findings for clinical studies and the mitigation strategy for the phase 1 clinical studies is presented in [Table 3.](#_bookmark68) The occurrence of clonic convulsion in one animal in the 1-week exploratory toxicity study in cynomolgus monkeys warrants the setting of an initial clinical exposure cap.

### Table 2 Exposure Ratio Between Toxicokinetic Values and the Anticipated Effective Exposure (Unbound Fraction)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study type** | **Sex** | **Dose (mg/kg)** | **Exposure ratio** | | | | **Study No.** |
| **Cmax,u** | | **AUC24,u** | |
| **First dose** | **Last dose** | **First dose** | **Last dose** |
| SD rats:  (non-GLP) Exploratory 1-week oral dose toxicity study | M  F | 3  3 | 4.1  7.5 | 2.6  5.9 | 2.0  3.6 | 1.6  1.9 | Project H-TX-  0014 |
| M  F | 10  10 | 17  27 | 20  35 | 9.5  17 | 17  27 |
| M  F | 30  30 | 42  95 | 125  94 | 35  105 | 93  103 |
| M | 100 | 236 | 888 | 333 | 1204 |
| F | 60 | 225 | 469 | 228 | 576 |
| SD rats:  4-week oral dose toxicity study | M  F | 1  1 | 0.9  2.5 | 1.4  3.8 | 0.5  1.1 | 0.7  1.3 | Project H-TX-  0003 |
| F | 3 | 8.2 | 10 | 4.0 | 5.7 |
| M  F | 10  10 | 15  32 | 21  50 | 11  29 | 19  36 |
| M  F | 30  30 | 54  90 | 90  127 | 46  91 | 107  149 |
| M | 60 | 105 | 252 | 105 | 326 |
| SD rats: additional  4-week oral dose toxicity study | M | 30 | 58 | 136 | 46 | 114 | Project H-TX-  0019 |
| M | 60 | 122 | 471 | 116 | 363 |
| SD rats:  Embryo-fetal development study | F | 3 | 10 | 12 | 3.7 | 5.4 | Project H-TX-  0009 |
| F | 10 † | 48 | 33 | 24 | 30 |
| F | 30 | 152 | 124 | 89 | 152 |
| Cynomolgus monkeys:  Safety pharmacology study | M | 1 | 2.0 | NA | 1.4 | NA | Project H-PT-  0004 |
| M | 3 | 11.4 | NA | 7.4 | NA |
| M | 10 | 73.1 | NA | 82.0 | NA |
| M | 30 | 164 | NA | 161 | NA |
| M | 60 | 140 | NA | 156 | NA |
| Cynomolgus monkeys:  (non-GLP) Exploratory 1-week oral dose toxicity study | M F | 3  3 | 9.2  21 | 17  48 | 5.3  8.6 | 13  16 | Project H-TX-  0015 |
| M  F | 10  10 | 30  91 | 95  195 | 22  90 | 66  154 |
| M  F | 30  30‡ | 192  179 | 373  529 | 235  273 | 505  443 |
| Cynomolgus monkeys: Single oral dose toxicity study | M  F | 30  30 | 232  46 | NA | 307  38 | NA | Project H-TX-  0002 |
| M  F | 60  60 | 229  161 | NA | 235  170 | NA |
| Cynomolgus monkeys:  4-week oral dose toxicity study | M  F | 3  3 | 4.4  3.6 | 14  8.3 | 3.7  2.7 | 9.4  5.7 | Project H-TX-  0004 |
| M F | 10  10 | 47  40 | 177  82 | 43  37 | 151  68 |
| M  F | 20  20 | 112  66 | 248  152 | 127  67 | 224  140 |
| NZW rabbits: Embryo-fetal development study | F | 1 | 0.8 | 0.9 | 0.1 | 0.2 | Project H-TX-  0010 |
| F | 3§ | 3.8 | 6.1 | 0.9 | 1.2 |
| F | 10† | 35 | 45 | 6.0 | 7.6 |

*Footnotes continued from previous page*

GLP: Good Laboratory Practice; NA: not applicable; NOAEL: no observed adverse effect level (underlined); NZW: New Zealand White

† NOAEL for fetus

‡ The dose level was decreased to 20 mg/kg for female from day 5.

§ NOAEL for dams,

The exposure ratio was calculated as follows:

The effective plasma concentration (Ceff) based on the pharmacology data in a cat model of acetic acid-induced irritated cystometry (0.03 mg/kg iv, 15min); 2.996 ng/mL

Ceff,u; 0.629 ng/mL (Ceff x fp,cat)

The effective AUC,u; 7.548 ng∙h/mL (Ceff,u x 12h) Exposure ratio (Cmax,u): TK Cmax x fp,animal / 0.629 Exposure ratio (AUC24,u): TK AUC24 x fp,animal / 7.548

(fp,animal: 0.19 for SD rats, 0.18 for cynomolgus monkeys, 0.25 for rabbits, 0.21 for cats)

### Table 3 Summary of Nonclinical Findings and Their Relevance to Human Usage

|  |  |  |
| --- | --- | --- |
| **Target organ** | **Potential Safety Concern (from nonclinical studies or from literature)** | **Relevance to Human Usage and Risk Mitigation Actions** |
| Central Nervous System | From animal studies: Decreases in locomotor activity, incomplete eyelid opening, slight flaccidity in abdominal tone, prone position, a low level of arousal, limp extensor thrust reflex, decreases in the escape response disappearance of proprioceptive positioning reaction, piloerection, and clonic convulsion.  Decreases in food consumption and body temperature.  From literature: potential for prolactin and cortisol increase, hallucinations, psychosis, cognitive effects, sedation, depression | Potential for several effects on central nervous system (including appetite) will be monitored by questionnaires, cognition test battery and AE reporting.  Body temperature will be monitored to investigate the potential for hypothermia. A Cmax exposure cap will be applied to minimize the risk for convulsion. |
| Vacuolar changes (suspected phospholipidosis) | Alveolar foam cells in the lung. Vacuolation in lymphocytes, kidney, epididymis and eye (1 rat) | Urine and serum di-22:6-BMP will be used as an exploratory marker for lysosomal effects that may progress to phospholipidosis. |
| Cardiovascular | Skin flush in rats.  Decreases in blood pressure and heart rate, prolongation of PR interval and QRS duration in cynomolgus monkeys.  From literature: potential for 5-HT2B related valvular heart disease. | Potential for skin flush will be monitored by adverse event reporting  Extensive ECG monitoring (incl. Holter and telemetry) will be used to assess the potential for cardiovascular effects. |
| Water balance | Increase in urinary volume and decrease in specific gravity in rats | Urine osmolarity, urine production and fluid intake will be monitored |
| Hematopoietic system | Decreases in erythrocytes, hemoglobin, and hematocrit. Increases in reticulocyte ratio, platelets, eosinophils and large unstained cell count | Standard monitoring of hematological parameters will be included in the phase 1 SAD and MAD studies. |
| Embryo-fetal development | No teratogenicity, but low body weight and low numbers of ossified sternebrae and sacral and caudal vertebrae, indicative of growth retardation resulting from maternal  toxicity in rats | When including women of childbearing potential, subjects need to be protected from pregnancy using appropriate measures of birth control. |
| Pharmacokinetics | More than dose proportional increase in PROJECT H plasma exposure was observed after single dose administration in rats and monkeys | During the SAD study, information of PROJECT H plasma concentrations of the previous dosing groups will be available prior to dose escalation. Besides safety, the dose escalation decision will be based on the available pharmacokinetic data and prediction of the exposures by modeling &  simulation. |

AE: adverse event; Cmax: maximum concentration; di-22:6-BMP: di-docosahexanoyl- bis(monoacylglycerol)phosphate; ECG: electrocardiogram; MAD: multiple ascending dose; PR: time from the onset of the P wave to the start of the QRS complex; SAD: single ascending dose

#### List of References

Abrams P, Schäfer W, Liao L, Mattiasson A, Pesce F, Spangberg A, et al. International Continence Society. Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies. Neurourol Urodyn. 2002;21(3):261-74.

Amacher DE Schomaker SJ, Burkhardt JE. The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. Food Chem. Toxicol.

1998;36:831-9.

Jost W, Marsalek P. Duloxetine: mechanism of action at the lower urinary tract and Onuf's nucleus.

Clin Auton Res. 2004;14(4):220-7.

Popp JA, Cattley RC. Hepatobiliary System. In: Fundamentals of Toxicologic Pathology, Haschek WM and Rosseaux, eds., Academic Press, Sandiego, 1998:127-51

Thor KB and Katofiasc MA. Effects of duloxetine, a combined serotonin and norepinephrine reuptake inhibitor, on central neural control of lower urinary tract function in the chloralose-anesthetized female cat. J Pharmacol Exp Ther. 1995;274:1014-24.

Wu KM, Farrelly JG. Preclinical development of new drugs that enhance thyroid hormone metabolism and clearance: Inadequacy of using rats as an animal model for predicting human risks in an IND and NDA. Am. J. Ther. 2006;13:141-4.