## 5.1.1 Safety Pharmacology

Safety pharmacology of PROJECT 6 was examined in *in vivo* studies in rats and dogs and *in vitro* APD assay using isolated papillary muscle of guinea pigs and hERG assay using recombinant cell lines expressing hERG ([Table 4](#_bookmark49)).

PROJECT 6 showed an increased spontaneous activity using Irwin’s method in rats, which can be attributed to the pharmacological activity of PROJECT 6. In dogs PROJECT 6 caused hyperactivity, salivation and clonic convulsion at 3 mg/kg or more and ataxic gait, hyperemia, defecation, loose stool, aggression, agitation and flush at the highest dose (10 mg/kg) tested. PROJECT 6 showed no effect on the respiration rate or hemoglobin oxygen saturation, and did not affect blood pressure, QT, QTc, or other intervals in the electrocardiogram in dogs. So, the *in vivo* results indicate that PROJECT 6 at dose levels up to 10 mg/kg, p.o. had no effect on the respiratory and cardiovascular system in rats and dogs, except for the increased heart rate in the telemetry study in dogs with 71% and 92% at 3 mg/kg and 10 mg/kg, respectively, which accompanied the hyperactivity observed in the animals. With respect to the *in vitro* studies, PROJECT 6 did not affect action potential duration in isolated papillary muscle of guinea pigs, and only slightly inhibited (14%) the hERG currents in HEK293 cells at the highest dose tested (1.5x10-6 mol/l), as compared to the inhibition of 87%, which was observed at a concentration of 1.0x10-7 mol/l of the positive control E-4031.

### Table 4 Summary of Safety Pharmacology Studies of PROJECT 6

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study No. [Reference]** | **Type of study** | **Species, Strain (M/F); Test**  **Model** | **Dose** | **Endpoints** | **Major Findings** |
| Project 6-PT-  0002 | hERG current (*in vitro*) | hERG-transfected HEK293 cells; Whole-cell patch-  clamp technique | 1.5x10-8,  1.5x10-7,  1.5x10-6 mol/l | Inhibition of hERG  current (IC50) | 1.5x10-6 M: slight inhibition (14.0%)  ≥1.5×10-7 M: No  effect |
|  |  |  | E-4031:  1.0x10-7 mol/l |  | E-4031: 87%  inhibition |
|  |  |  | (positive |  |  |
|  |  |  | control) |  |  |
| Project 6-PT-  0003 | Action potential  duration (*in vitro*) | Papillary muscles  (n=5) isolated from Hartley | 0.95x10-8,  1.02x10-7,  1.11x10-6 | Resting  membrane potential, action | No effects on any parameters |
|  |  | Guinea pig; Glass | mol/l | potential |  |
|  |  | electrode |  | amplitude, |  |
|  |  | technique | E-4031:  1x10−7 mol/l | dV/dt max,  action potential |  |
|  |  |  | (positive control) | duration (APD30 and APD90) |  |
| Project 6-PT- | Central nervous | Rat, SD, | 0.1, 1, 10, 100 | General activity | 10: ↑ spontaneous |
| 0001 | system (*in vivo*) | 6M; Modified | mg/kg, po | and behavior | activity and |
|  |  | Irwin’s method |  |  | salivation |
|  |  |  |  |  | ≥10:↑ touch escape |
|  |  |  |  |  | 100: ↓urination |
|  |  |  |  |  | frequency |
| Project 6-PT-  0004 | Central nervous, cardiovascular and respiratory system (*in vivo*) | Dog, beagle, 4M; Telemetry (unanesthetized) | 1, 3, 10  mg/kg, single dose, po  Astemizole: 20 mg/kg (positive control) | General activity and behavior, body temperature, blood pressure, heart rate, ECG, Respiration rate, blood gases, blood- electrolyte  concentration | ≥3: Hyperactivity, salivation, clonic convulsion; ↑ heart rate  10: Ataxic gait, hyperemia, defecation, loose stool, aggression, agitation and flush |

## Toxicology

### Table 10 Nonclinical Safety Studies of PROJECT 6

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study No [Reference]** | **Type of Study** | **Species, Strain** | **No. of**  **Animals/ Sex/Dose** | **Route** | **Doses (mg/kga)** |
| Project 6-TX-0001 | Single-dose | Rat, SD | 5M, 5F | Oral  gavage | 500, 1000, 2000 |
| Project 6-TX-0002 | Single-dose | Dog, Beagle | 1M, 1F | Oral  gavage | 100, 500 |
| Project 6-TX-0004 | 4-week repeat- dose | Rat, SD | 10M, 10F\*  \*: 5M&5F were added in 0 and  100 mg/kg for recovery | Oral gavage | 0, 0.1, **1**, 10, 100 |
| Project 6-TX-0006 | 4-week repeat- dose | Dog, Beagle | 3M, 3F\*  \*: 3M&3F were added in 0 and 30 mg/kg for recovery | Oral gavage | 0, 0.3, 1, **3**, 30 |
| Project 6-TX-0007 | Reverse mutation test | *S. typhimurium* (TA100, TA98, TA1535, TA1537)  *E.coli* (WP2*uvrA*) | NA | NA | 39.1-5000  μg/plate |
| Project 6-TX-0008 | Chromosomal aberration test | Chinese hamster lung fibroblast cells | NA | NA | 6h: 100-300  μg/ml 24h: 10-30  μg/ml |
| Project 6-TX-0010 | Reproductive and  developmental | Rat, SD | 19-20F | Oral  gavage | 0, 10, 30, 100 |
| Project 6-TX-0012 | Reproductive and  developmental | Rabbit, NZW | 21-22F | Oral  gavage | 0, 1, 3, 10 |
| Project 6-TX-0013 | Fertility and early  embryonic development | Rat, SD | 20M | Oral gavage | 0, 1, 10, 100 |
| Project 6-TX-0014 | Fertility and early embryonic  development | Rat, SD | 20F | Oral gavage | 0, 1, 10, 100 |

a Unless otherwise specified. For repeat-dose toxicity studies, the highest NOAEL is underlined; NA: not applicable

### Single Dose Studies

A single dose oral toxicity study with PROJECT 6 has been performed in rats (500, 1000 and 2000 mg/kg) and in Beagle dogs (100 and 500 mg/kg) (see [Table 11](#_bookmark64)). The approximate lethal dose levels of PROJECT 6 were 1000 mg/kg in male and female rats, 500 mg/kg in male dogs and more than 500 mg/kg in female dogs.

### Table 11 Summary of Single-Dose Nonclinical Safety Studies of PROJECT 6

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species,**  **Strain** | **No./Sex/**  **Dose** | **Route** | **Dose**  **(mg/kg)** | **Death** | **Major Findings** |
| Project 6-TX-  0001 | Rat, SD | 5M, 5F | oral gavage, observation for 14 days | 500,  1000,  2000 | 2M; 4F  5M; 4F | Approximate lethal dose: 1000 mg/kg  CNS changes:  ≥500: ↑ spontaneous movement, ataxia, prone/lateral position,  ≥1000: hypothermia, bradypnea,  GI tract changes:  ≥500: watery stool  ≥1000: dark red focus (erosion) in glandular stomach in dead animals  Other changes:  ≥500: salivation, ↓ body weights, lacrimation |
|  |  |  |  |  |  | Approx. lethal dose 500 mg/kg (M); >500 mg/kg (F)  CNS changes:  ≥100: restlessness; F: staggering gait, tonic convulsion  500: staggering gait, tonic convulsion, aggressive behavior; F: excitement Other changes:  ≥100: stool containing test article-like material, ↓ body weight  500: ↑ AST; F: ↑ A/G ratio |
| Project 6-TX-  0002 | Dog, beagle, | 1M, 1F | oral gavage, observation for 14 days | 100,  500 | 1M (day 2) | **Death animal:**  Blood changes:  ↑ RBC, hemoglobin, hematocrit, WBC, neutrophils, ALP, sodium, chloride, total protein and albumin, ↓ lymphocyte and potassium CNS changes:  ↓ spontaneous movement, mydriasis, twitch and prone position, degeneration/ necrosis of nerve cells in the hippocampus and cerebral cortex, perivascular/meningeal cell infiltration or hemorrhage in the cerebral cortex  GItract changes:  vomiting containing test article- like material; dark red focus in stomach,  Heart changes: hydropericardium and dark red  focus in endocardium, |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species,**  **Strain** | **No./Sex/**  **Dose** | **Route** | **Dose**  **(mg/kg)** | **Death** | **Major Findings** |
|  |  |  |  |  |  | myocardial degeneration/ necrosis, fibrinoid necrosis of arterial wall in the heart Other changes:  dark red discoloration in the lung, degeneration/necrosis of  cortical cells in the adrenal |

### Repeated Dose Studies

Repeated dose toxicity studies have been performed in rats and dogs up to 4-week treatment (see [Table 12](#_bookmark66)). In the 4-week studies, 1 mg/kg/day and 3 mg/kg/day are the NOAELs for rats and dogs, respectively.

Rats

A 4-week oral toxicity study in rats was performed at dose levels of 0.1, 1, 10 and 100 mg/kg/day. Target organs of PROJECT 6 in rats were the CNS, liver and urinary system. Small treatment related findings were also observed in the hematopoietic system and salivary glands. In a small number of rats (<25%) treated with 0.1 and 1 mg/kg/day an increase in spontaneous movement was observed. In the 10 mg/kg/day and 100 mg/kg/day dose groups, all animals showed an increase in spontaneous movement, which also was sustained relatively longer than in the animals treated with lower doses, but the incidence decreased with repeated administrations in the 100 mg/kg dose group. Eating-like behavior was occasionally observed at pre-dosing in the animals treated with 10 mg/kg/day or more and muscle weakness and salivation was found in animals treated with 100 mg/kg/day. Therefore the NOAEL was estimated to be 1 mg/kg for both sexes.

In the animals treated with 100 mg/kg/day some effects on the liver function was suggested, since increased weight, hypertrophy of the centrilobular hepatocytes and decreased vacuolation in the periportal hepatocytes in the liver were noted. The findings in the animals treated with 10 mg/kg/day or less and the increased ALT in females were considered not to be toxicologically relevant, since values were within the range of the historical control data and no histopathological changes were noted.

Other findings included a decrease in urine volume, excretion of chloride and an increase in urinary osmolality, BUN and creatinine in the 100 mg/kg/day group, however, no histopathological changes were observed in the kidney. Also minimally decreased erythrocyte parameters (5% decrease in hemoglobin and hematocrit) in animals treated with 10 mg/kg/day or more were suspected to be test article-related, since increased extramedullary hematopoiesis in the spleen was also noted in these animals.

Dogs

A 4-week oral dose toxicity of PROJECT 6 was investigated in beagle dogs at daily doses of 0.3, 1, 3 and 30 mg/kg/day. The target organ was identified to be the CNS. Observation of general condition revealed a series of behavioral changes that reflect the pharmacological effect of the test article on the CNS, which was exclusively seen in the 30 mg/kg group during first days of treatment. The changes observed included restlessness, excitement, tonic convulsion, staggering gait, aggressive behavior and a decrease in spontaneous movement. These changes occurred simultaneously every day for several days after the start of treatment and diminished thereafter to occasional occurrence. Excitement and/or restlessness were also observed in 2 males and 1 female dog that were treated with 3 mg/kg/day, mostly during the first 5 days of treatment. However, excitement and restlessness were not considered to be an adverse effect. In the 0.3 and 1 mg/kg/day groups, no changes were observed. Therefore the NOAEL was estimated to be 3 mg/kg for both sexes. Based on the decreased incidence of the behavioral changes with repeated administration it appears that the animals rapidly tolerated the exaggerated pharmacological effects of the test article.

TK data of the 4-week repeated dose toxicity studies in rats and dogs are shown in [Table 6](#_bookmark54).

### Table 12 Summary of Nonclinical Repeated-Dose Safety Studies of PROJECT 6

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species,**  **Strain** | **No./Sex/**  **Dose** | **Route** | **Dose**  **(mg/kg)** | **Death** | **Major Findings** |
| Project 6-TX-  0004 | Rat, SD | 10M, 10F\*  \*: 5M&5F  were added in 0 and  100 mg/kg for recovery | oral gavage, recovery for 4 weeks | 0  0.1  **1**  10  100 | No deaths | CNS changes:  ≥0.1: ↑ spontaneous movement  ≥10: eating-like behavior 100: muscle weakness Liver changes:  100: liver weight, hypertrophy of centrilobular hepatocytes and  ↓ vacuolation in the periportal hepatocytes; F: ↑ ALT Urinary system changes:  ≥10: M: ↓ urine volume and urine chloride, ↑ urine osmolality  100: F: ↑ BUN, creatinine and water intake  Other changes:  ≥0.1: minimal ↓ hemoglobin, hematocrit  10: ↑ food consumption and salivary gland weights, hypertrophy of acinar cells in the submandibular glands, F: hypertrophy of acinar cells in the sublingual glands  ≥10: extramedullary hematopoiesis in the spleen  100: salivation, M; ↓ plasma chloride, ↑ pituitary weight, |
| Project 6-TX-  0006 | Dog, beagle, | 3M, 3F\*  \*: 3M&3F  were added in 30 mg/kg for recovery | oral gavage, recovery for 4 weeks | 0,  0.3,  1,  **3**,  30 | No deaths | CNS changes:  ≥3: restlessness; M: excitement 30: tonic convulsion, staggering gait, aggressive behavior, excitement and decrease in spontaneous movement  Other changes:  30: M:↑ kidney weights, F: ↑ ALT |

The NOAELs are bold and underlined.

### Reproductive Toxicity

The results of the fertility and early embryonic development studies in male and female rats [(Table 13](#_bookmark68)) showed increased spontaneous movement in female rats treated with 1 mg/kg or more and males treated with 10 mg/kg or more. A transient decrease in food consumption, without an associated decrease in bodyweight, was noted for males treated with 100 mg/kg; however, an increase in food consumption and bodyweight were noted for females receiving 10 mg/kg or more. No other test-article related effects were observed in terms of general toxicity. There were no effects on the indices of fertility (copulation index, numbers of

corpora lutea or spermatogenesis) or early embryonic development (such as implantation) in males or females at any dose level examined. The NOAELs for general toxicity were 10 mg/kg and 1 mg/kg in males and females, respectively. For fertility and early embryonic development in males and females the NOAEL was 100 mg/kg in both species.

The results of the embryo-fetal development studies in rats and rabbits ([Table 13](#_bookmark68)) showed increased spontaneous movement in the rats treated with 10 mg/kg or more and a decrease in body weight and food consumption in the rats treated with 100 mg/kg. In the rabbits treated with 3 mg/kg or more decreased body weight and food consumption were observed. One dam treated with 10 mg/kg showed an abortion. External, visceral and skeletal examinations in the foetuses did not show any abnormalities in rabbits. In rats a decreased number of ossified metatarsi was observed at 30 mg/kg or higher. Therefore the NOAEL for the dams was 30 mg/kg in rats and 1 mg/kg in rabbits and the NOAEL for the embryo-fetal development was 10 mg/kg for both species.

### Table 13 Summary of Nonclinical Reproductive Toxicity Studies of PROJECT 6

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species,**  **Strain** | **No./Sex/**  **Dose** | **Route** | **Dose**  **(mg/kg)** | **Death** | **Major Findings** |
| Project 6-TX-  0010 | Rat, SD | 19-20F | oral gavage, days 7 to  17 of gestation | 0,  **10$**,  **30#**,  100 | No deaths | Dams  ≥10:↑ spontaneous movement 100: ↓ body weight and food consumption  Fetuses  ≥30: ↓ number of ossified metatarsi  100: ↓ body weight |
| Project 6-TX-  0012 | Rabbit, New Zealand white | 21-22F | oral gavage, days 6 to  18 of gestation | 0,  **1**#,  3,  **10**$ | No deaths | Dams  ≥3: ↓ body weight and food consumption  10: Abortion (1F) Fetuses  No effects on fetuses in any doses |
| Project 6-TX-  0013 | Rat, SD | 20M | oral gavage, for 9 weeks prior to  mating | 0  1  **10+**  **100&** | No deaths | ≥10: ↑ spontaneous movement  ≥100: ↓ food consumption No effects on fertility, copulation or implantation |
| Project 6-TX-  0014 | Rat, SD | 20F | oral gavage, for 9 weeks prior to mating | 0  **1+**  10  **100&** | No deaths | ≥1: ↑ spontaneous movement  ≥10: ↑ body weight and food consumption  No effects on fertility or early embryonic development |

The NOAELs are bold and underlined.

# - NOAEL to the dams $ - NOAEL to the fetuses

+ - NOAEL for general toxicity & - NOAEL for fertility and early embryonic development

### Genotoxicity (Mutagenicity)

PROJECT 6 was demonstrated to be not genotoxic in the in vitro tests (see [Table 14](#_bookmark70)).

### Table 14 Summary of Nonclinical Genotoxicity Studies of PROJECT 6

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study No.** | **Study** | **Species, Strain,**  **Dosing particulars** | **Dose** | **Major Findings** |
|  |  | *S. typhimurium* | 39.1-5000 μg/plate | No increase in revertant colonies |
| Project 6-TX- | Reverse | (TA100, TA1535, |  |  |
| 0007 | mutation test | TA98, TA1537) |  |  |
|  |  | *E.coli* (WP2*uvrA*) |  |  |
|  |  | Chinese hamster | 6h, -S9: 100-200 | No increase in chromosomal |
|  |  | lung fibroblast cells | μg/mL | aberration cells |
| Project 6-TX-  0008 | Chromosomal aberration test | Treatment for 6h with or without  metabolic activation | 6h, +S9: 100-300  μg/mL  24h, -S9: 10-30 μg/mL |  |
|  |  | system (S9) and for |  |  |
|  |  | 24h without S9 |  |  |

## Integrated Discussion of Nonclinicial Data

Based on preclinical data, it seems justified that PROJECT 6 enters clinical development for the treatment of AD and the cognitive symptoms of schizophrenia.

PROJECT 6 acts as a blocker at BEC1 K+ channels, which are located on GABAergic interneurons in the forebrain. Administration of PROJECT 6 results in inhibition of these interneurons, which leads to an excitation of principal cholinergic and dopaminergic neurons. Thus PROJECT 6 administration results in a moderate increase in PFC dopamine and ACh efflux, which is a potential mechanism of action through which PROJECT 6 enhances cognition in AD and counteracts schizophrenic symptoms in schizophrenia. *In vitro* data imply that PROJECT 6 may also induce neurochemical differences in the hippocampus, a structure that is well known for its role in learning and memory. The fact that PROJECT 6 modulates the forebrain cholinergic system could implicate a functional interaction with acetylcholinesterase inhibitors such as donepezil. However, this interaction is not believed to be significant at clinically effective doses.

PROJECT 6 was effective in both short-term (working memory) tasks such as spontaneous alternation as well as long-term retention such as measured in the inhibitory avoidance task. Therefore, the animal data would suggest that PROJECT 6 can be used in a wide spectrum of cognitive impairment. PROJECT 6 was also effective in animal models that represent both traditional (methamphetamine-induced hyperlocomotion) and SGA-like (PCP-induced hyperlocomotion and disruption of PPI) psychotic profiles of schizophrenia. In addition to negative symptoms, PPI is also associated with positive and cognitive (deficits of information processing) symptoms. Collectively, the data provide some evidence that PROJECT 6 may be effective in positive and negative symptoms of schizophrenia, although more convincing evidence was obtained on treatment for the cognitive symptoms (PPI and other animal models of cognition). Effective doses ranged from 0.01-0.3 mg/kg, depending on which model was used. Generally, higher doses were required in animal models of psychosis or in pharmacological challenge studies and the lower doses were sufficient in the aged rat models. This may implicate that PROJECT 6 modulates behavior in models of cognition and psychosis through different neurotransmitter systems, although the lower doses narrowly missed significance in the schizophrenic models. Dosing in the aged rat model may be most representative of cognition in the clinic since it does not involve pharmacological treatment. Granted, like the other models, the aged rat model lacks the structural and molecular changes seen in the brain of AD patients. Importantly, none of the behavioral data were compromised by an effect on other measures such as locomotor activity. The shape of the dose response curves depended on the model that was used and ranged from linear to bell-shaped.

Asymptotic effects were seen with higher doses in some models.

In line with convincing pharmacological activity, PROJECT 6 is rapidly taken up into the brain. In addition, the compound has a good safety profile, but some caution should be used in interpreting the data, as outlined below. In both rats and dogs PROJECT 6 has a variable dose- dependent bioavailability, which increases with dose and is probably caused by saturable pre- systemic drug elimination (e.g. first-pass effect). Upon administration, PROJECT 6-related

radioactivity was rapidly absorbed and distributed to different tissues including the brain, the targeted organ for PROJECT 6 pharmacological activity. No accumulation of PROJECT 6 in the brain was observed in the tissue distribution study. Tissue distribution studies with labeled PROJECT 6 in rats revealed relatively high concentrations in the eyeball from which it was slowly eliminated (tissue elimination t1/2: 537.2 hours). Autoradioluminograms showed binding to melanin-rich areas of the eyeball suggesting that PROJECT 6 and/or its metabolites to have affinity for melanin. However, no difference in tissue eliminated was noted between pigmented and non-pigmented skin. In single dose PK study in rats, the exposures of the PROJECT 6 were 4-5 times higher in the brain than in the plasma with similar t1/2 values between plasma and brain. These findings are supported by the results of another exposure study in aged rats (Project 6-PH-0213). Together with the data that a good brain penetration was also observed in rhesus monkeys (Project 6-PH-0211), it is expected that an exposure pattern favors brain will be observed in human as well.

The selection of rat and dog as safety species is supported by the *in vitro* metabolite fingerprinting study. All PROJECT 6 metabolites detected in human liver microsomes were also formed in those of rats, rabbits, and dogs and there are no indications for human-specific PROJECT 6 metabolites.

The safety profile of PROJECT 6 indicates that the CNS is the first target organ. In the repeated dose toxicity studies both rats and dogs showed excitatory changes in behavior already at low doses (0.1 mg/kg in rats and 3 mg/kg in dogs). At the highest dose levels all animals showed CNS related events, such as eating-like behavior (animals that act like they are eating) and muscle weakness in rats. In dogs convulsions, aggressive behavior and decrease in spontaneous movement were observed. Convulsions were also observed in dogs at a single dose of 3 mg/kg or more in the safety pharmacology study (Project 6-PT-0004), therefore convulsions could be a dose-limiting side effect already at single dose. The lowest Cmax unbound (6 ng/mL) at 1 mg/kg (NOAEL from the safety pharmacology study) in dogs ([Table](#_bookmark72) [15](#_bookmark72)) is 80 times higher than the Cmax unbound at which pharmacological effect was observed in the most sensitive pharmacological model, being the aged rat model. Therefore, severe CNS side effects are not expected when PROJECT 6 will be administered to healthy volunteers or patients up to dose levels that demonstrate a pharmacological effect. However, when doses are escalated above the pharmacological effective concentration, CNS related adverse events have to be closely monitored (restlessness, agitation, changes in mood) to pick up early adverse effects on the brain in order to prevent the occurrence of convulsions (see section 7).

### Table 15 Compilation of systemic exposure data of PROJECT 6 at NOAELs

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species, Strain** | **Sex (M/F)** | **Dose (mg/kg**  **)** | **HED**  **(mg/ 60kg)** | **Cmax (ng/mL)** | | **AUC0-24h**  **(ng.h/mL)** | | **Remarks** |
| First  dose | Last  dose | First  dose | Last  dose |
| Project 6-PT-0004  (single dose) | Beagle Dog | M | 1 | 32.4 | 121.56  (6.08) |  | 205.37  (10.27) |  | Clonic convulsions at 3  mg/kg |
| Project 6-TX-  0004  (4 weeks) | Rat, SD | M | 1 | 9.6 | 27.65  (1.38) | 28.96  (1.45) | 79.39  (3.97) | 141.47  (7.07) | NOAEL based on eating like behavior and extramedullary hematopoiesis at  10 mg/kg |
| F |  |  | 50.91  (2.55) | 56.43  (2.82) | 130.50  (6.53) | 143.60  (7.18) |
| Project 6-TX-  0006  (4 weeks) | Beagle Dog | M | 3 | 97.2 | 206.41  (10.32) | 142.98  (7.15) | 818.20  (40.91) | 395.23  (19.76) | NOAEL based on tonic convulsion, staggering gait, aggressive behavior, excitement and decrease in spontaneous movement at 30  mg/kg |
| F |  |  | 287.69  (14.38) | 178.80  (8.94) | 648.02  (32.40) | 415.30  (20.77) |
| Project 6-PH-  0207  Single dose  Passive avoidance test | Aged Rat, Fisher F344 | M | 0.0313 | 0.30 | 1.5#  (0.075) |  | 3.88#  (0.194) |  | Significant improvement in retention trial latency |

\* Free-fraction exposure level between brackets is based on the results from Project 6-ME-0009, which was conducted to determine the in vitro plasma protein binding of PROJECT 6. Values obtained were 97% in mice; 95% in rats; 97% in rabbits; 95% in dogs; 96% in cynomolgus monkeys and 96% in humans.

# Exposure extrapolated from PK data of study Project 6-ME-0004, assuming linear PK.

In the safety pharmacology studies, slight inhibition (14%) of the hERG current was observed at the highest concentration tested (1.5x10-6 M). Furthermore, no effects were observed on the APD and no QT or QTc-prolongation was observed in dogs treated up to 10 mg/kg. PROJECT 6 did increase the heart rate in the telemetry study in dogs with 71% and 92% at 3 mg/kg and 10 mg/kg, respectively, which accompanied the hyperactivity observed in the animals.

There was a minimal change in Hb and HCT in rats at 0.1 mg/kg or more, which was accompanied by an increased extramedullary hematopoiesis in the spleen at doses of 10 mg/kg or higher. Since no decrease was observed in RBC, MCV, MCH, MCHC or reticulocytes, these changes are not considered to be a clinically relevant adverse finding and normal hematopoietic tests are adequate to detect if changes occur in the clinic.

In rats, increased ALT, liver weight, hypertrophy of centrilobular hepatocytes and decreased vacuolation in the periportal hepatocytes was observed at the highest dose (100 mg/kg).

Similar changes were not observed in dogs after 4-weeks treatment. It is considered that the changes in rats, which were reversible after the treatment period, were adaptive changes linked to the elimination process of high dose of PROJECT 6, as opposed to a toxic change.

Therefore, normal liver function tests are adequate to detect changes, if any, in liver function in volunteers or patients treated with PROJECT 6.

In rats, a decrease in urine volume, excretion of chloride and an increase in urinary osmolality, BUN and creatinine was observed in the 100 mg/kg/day group. In dogs, an increase in kidney weight was observed in males treated with 30 mg/kg/day. However, in both species no histopathological changes were observed in the kidney. Therefore, normal kidney function tests are adequate to detect changes.

The side effect profile in dogs and rats showed central stimulant effects, but the overall picture is not completely in line with stimulation of the cholinergic, or dopaminergic system seen in the microdialysis study in rats. The combination of agitation, hypothermia, tachycardia and convulsions, is also observed with modulation of GABAA receptors in specific brain areas such as rostral ventral medulla and dorsomedial hypothalamus in rats [i, ii]. The expression profile of BEC1 in the brain is not entirely congruent with these findings. Therefore, currently there is no clear understanding of the mechanism of the central stimulating effects of PROJECT 6, which warrants a cautious approach in FIM with this drug.

Overall, the preclinical toxicity package, which consists of single and repeat dose studies in both rats and dogs, reproductive studies in rats and rabbits and *in vitro* genotoxicity assays, does not preclude further development. Specifically, the current dataset supports clinical studies up to 4-weeks of treatment. Furthermore, since no toxicity was observed in dams, and no significant effects were observed on the female reproductive system in the 4-week repeat dose studies, the dataset allows the inclusion of women of childbearing potential, provided that precautions are taken (*i.e.* barrier contraceptives) at the appropriate time. In spite of the relatively favorable safety profile of PROJECT 6, caution should be taken with dosing in clinical trials due to high inter-individual variability, which is associated with the dose- dependent bioavailability. Also, the dose response curves in preclinical models may indicate that the therapeutic dose-range for treating AD may be bell-shaped.

The combined data yields a good safety profile with good pharmacological activity and rapid absorption into the brain. Overall, the preclinical data is more convincing in showing potential efficacy for AD and cognitive dysfunction of schizophrenia rather than positive symptoms of schizophrenia.

1. Menezes, R.C., Fontes, M.A. Cardiovascular effects produced by activation of GABA receptors in the rostral ventrolateral medulla of conscious rats. Neuroscience 2007; 144: 336- 343.
2. Cao, W.H., Fan, W., Morrison, S.F. Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience 2004; 126: 229-240.