## Safety Pharmacology

PROJECT S has been assessed in primary and secondary pharmacology studies, as well as safety pharmacology studies using Project S; a tabulated overview of these studies may be found in (EOTT 1.2 and 1.3).

The nonclinical safety program included safety pharmacology studies in human cells, guinea pigs, rats and dogs: the potential to affect hERG current on channel-transfected HEK293 cells; potential effects on action potential duration (APD); and potential effects on the central nervous, cardiovascular and respiratory systems.

Results of the nonclinical safety pharmacology studies indicate the following:

* Project S did not affect the hERG current in hERG transfected HEK293 cells at concentrations up to 1 µmol/L. At 10 µmol/L, Project S suppressed the hERG current by approximately 44% (Study Project S-PT-0002).
* Project S did not affect the action potentials in isolated guinea pig papillary muscles at concentrations up to 1 µmol/L. Project S shortened the action potential duration at 30% repolarization (APD30) and prolonged the action potential duration between 90% and 30% repolarization (APD30-90) at 10 µmol/L (Study Project S-PT- 0003).
* In dogs, Project S had no effect on the central nervous, cardiovascular or respiratory systems at 3 or 10 mg/kg. At 30 mg/kg, decreases in group mean values of　blood concentrations of K+ and Ca2+ were noted. The animal with the highest plasma drug concentration showed vomiting, inability to stand, analgesia, clonic convulsion, loss of righting reflex, leaning, loose stool, staggering, increases in body temperature, blood pressure, heart rate and respiration rate, prolongation in QTc interval and decreased blood pH and blood gas of PaCO2. The animal with the second highest plasma drug concentration showed vomiting (Study Project S-PT-0004).
* In rats, Project S had no effect on the CNS up to 100 mg/kg. Decreased spontaneous activity, body tone, abdominal tone and salivation were observed at 300 mg/kg (Study Project S-PT-0001).

### Anticonvulsive Effect in Animal Model of Seizure

The effect of PROJECT S on PTZ-induced seizure threshold in rats was evaluated to assess pro- and/or anti-convulsant activities. Subcutaneous injection of PTZ at 50 mg/kg induced clonic convulsions in 90% of the treated rats. Pretreatment with PROJECT S (0.3 to 30 mg/kg, po)

30 minutes before the injection of PTZ reduced the rate of seizure incidence (Project S-PH-1006) [[Table 1](#_bookmark22)]. PROJECT S at doses of 0.3, 3 and 30 mg/kg did not induce proconvulsant activity at subconvulsant doses of PTZ (30 mg/kg).

### Table 1 Assessment of Pro- and/or Anti-convulsant Activities in Rat PTZ-induced Seizure Test

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | |  |  | |
| **ASP9226 dose (mg/kg, po)** | **PTZ dose (mg/kg, sc)** |  |  |  | **Incidence (%)** |
| **No. of animals**  **(evoked / tested)** | | |
| 0 | 30 | 0 / 10 | | | 0 |
| 0.3 |  | 0 / 10 | | | 0 |
| 3 |  | 0 / 10 | | | 0 |
| 30 |  | 0 / 10 | | | 0 |
| 0 | 50 | 9 / 10 | | | 90 |
| 0.3 |  | 8 / 10 | | | 80 |
| 3 |  | 6 / 10 | | | 60 |
| 30 |  | 6 / 10 | | | 60 |

PTZ: pentylenetetrazole; po: by mouth; sc: subcutaneous.

Seizures were evoked by 30 or 50 mg/kg (sc) PTZ in rats. PROJECT S or vehicle was administered orally 30 minutes before the injection of PTZ.

## Toxicology

A toxicology program was conducted to support the use of PROJECT S in clinical studies with treatment for up to 13 weeks. The toxicology program included single- and repeat-dose studies in rats and dogs, genotoxicity studies, male and female fertility studies in rats, and embryo-fetal developmental toxicity studies in rats and rabbits. In addition, intravenous infusion studies were conducted in rats and dogs in order to investigate conditions when　convulsions occur. There were also some preliminary non-pivotal (not definitive) studies performed; in some of these, AS203Project S-TA (D-tartrate of PROJECT S) was used because the studies were performed before the final salt form (L-tartrate) was fixed.

A tabulated overview of these studies may be found in EOTT 3.1.

### Single-dose Toxicity

Tabulated overviews of single-dose toxicity studies may be found in EOTTs 3.1 and 3.5. In addition, single oral dose toxicokinetic bridging studies with PROJECT S were conducted in rats and dogs, to bridge the two salt forms and a bolus iv study in dogs was conducted to evaluate clinical signs. These studies are tabulated in EOTT 3.1, and not described here.

### Rats

In a study in rats (Study Project S-TX-0001; EOTTs 3.1 and 3.5), major symptoms in males at

≥ 1000 mg/kg and in females at ≥ 100 mg/kg were clonic convulsion and abnormal position, as well as a decrease in spontaneous activity and ataxic gait in males at ≥ 300 mg/kg and in females at ≥ 100 mg/kg. The approximate lethal dose (LD) level in rats was 1000 mg/kg of PROJECT S for males and 300 mg/kg for females.

### Dogs

In a study in dogs (Study Project S-TX-0002; EOTTs 3.1 and 3.5), there was no toxicologically significant finding at 30 mg/kg. At 100 and 200 mg/kg, all animals showed tonic convulsion, resulting in death in 1 male at 100 mg/kg and in 1 female at 200 mg/kg. The other surviving male and female also showed vomiting, decrease in locomotor activity, staggering gait, lateral position or salivation. In the 100 mg/kg female, prolongation of QT and QTc was noted. The approximate LD level in dogs was 100 mg/kg.

After a single intravenous administration at 3 mg/kg to dogs (Study Project S-TX-0018; EOTT 3.1, and 3.5), no vomiting occurred and no abnormalities were found. The plasma concentration at 0.1 hour after dosing (C0.l h) ranged from 664.3 to 984.6 ng/mL.

### Repeat-dose Toxicity

Exploratory oral repeated dose range-finding studies were conducted with AS203Project S-TA (D-tartrate of PROJECT S) in rats and dogs. At the start of Good Laboratory Practices (GLP) toxicity studies, the salt form was changed from D-tartrate to L-tartrate, and definitive 4- and 13-week oral repeated dose toxicity studies in both rats and dogs were conducted with Project S.

### Repeat-dose Toxicity in Rats

### Exploratory 1-week Oral Dose Range Finding Study

In a 1-week oral dose range finding (exploratory) study in rats (Study Project S-TX-0015; EOTT 3.1 and 3.6), symptoms at 100 mg/kg were salivation, prone position, blepharophimosis and ataxic gait. In males, there was hypomyotonia; in females, there was decreased locomotor activity, increased aspartate aminotransferase (AST) and increased mucus secretion in the fundus of stomach. At 300 mg/kg, 2 females died 0.5 or 1.5 hours　after dosing on day 1; the remaining 2 females were sacrificed in extremis 3 hours after dosing. In addition to the observations made at 100 mg/kg, the following were observed: In both sexes, there was mydriasis. In males, suppression of body weight gain, decrease in total protein and increased relative weight of the liver and centrilobular hypertrophy of hepatocytes were observed. In females, hypomyotonia, lateral position, bradypnea, rigidity of limbs, lacrimation, gnawing motion, trauma (nose), increases in alanine aminotransferase (ALT) and glucose, decreases in sodium and chloride, sporadical red spot in the fundus of stomach, shortening of villi in the duodenum and atrophy in the red and white pulp of the spleen were observed. The plasma unchanged drug concentrations after single and 1-week dosing regimens reached Cmax rapidly and showed a tendency to increase with increased dose except for the 300 mg/kg male on single dosing; plasma unchanged drug concentration was higher in females than in males. No differences were observed between single and repeated dosing.

### Definitive 4-week Oral Toxicity Study

In a 4-week repeat-dose oral toxicity study in rats (Study Project S-TX-0004**;** EOTTs 3.1, 3.3, 3.4 and 3.7.1), clonic convulsion, ataxic gait, abnormal position (prone or lateral position), decreased spontaneous activity, salivation, mydriasis, suppression of body weight gain, low food consumption, high urine volume with low specific gravity, high leukocyte count (mainly due to lymphocyte count), high total cholesterol and high kidney weight were observed in both sexes at 300 mg/kg. In males at 300 mg/kg, there was shortened prothrombin time and activated partial thromboplastin time and elevated serum inorganic phosphorus, calcium and potassium; in females at 300 mg/kg, 3 animals died within 2 hours after dosing on day 1,

20 or 22 of dosing; high adrenal and ovary weights were observed in survivors. At

1000 mg/kg, almost all animals died, mainly within 2 hours after dosing by day 8 of dosing. Clonic convulsion, ataxic gait, abnormal position (prone or lateral position), decrease in spontaneous activity, salivation, mydriasis, emaciation, decrease in stool volume, trace of reddish rhinorrhea, perineal region soiled by urine, decrease in body weight and low food consumption were observed.

Changes considered related to drug metabolizing enzyme induction included hypertrophy of the centrilobular hepatocytes in ≥ 30 mg/kg males and in ≥ 100 mg/kg females; high liver weight in ≥ 100 mg/kg males and in 300 mg/kg females; high total cholesterol observed in males and females at 300 mg/kg and high inorganic phosphorous, calcium and potassium in males. All findings noted during the dosing period in 300 mg/kg females resolved after drug withdrawal. The NOAEL was 100 mg/kg/day PROJECT S. In toxicokinetics, Cmax increased less than dose-proportionally in both sexes on days 1, 14 and 28 of dosing, except at 30, 100 and 1000 mg/kg on day 1 of dosing. Cmax at 30 and 100 mg/kg on day 1 of dosing increased more than dose-proportionally and Cmax at 1000 mg/kg on day 1 of dosing was similar to that at 300 mg/kg. Area under the plasma concentration-time curve from 0 to 24 hours after dosing (AUC24) increased more than dose-proportionally in both sexes on days 1, 14 and 28 of dosing. Cmax and AUC24 were higher in females than in males on days 1, 14 and 28 of dosing, except for the Cmax at 300 mg/kg on day 1 of dosing, which was similar in males and　females. Cmax and AUC24 were almost constant or slightly decreased during the dosing period (EOTT 3.5).

### Definitive 13-week Oral Toxicity Study

When PROJECT S was administered to rats for 13 weeks (Study Project S-TX-0021; EOTTs 3.1, 3.3, 3.4 and 3.7.3) no test article-related changes were observed in males at 10 mg/kg or in females at 10 or 30 mg/kg.

At 30 mg/kg, low triglycerides were observed in males.

At 100 mg/kg, low triglycerides, high total cholesterol and calcium, dark discoloration of the lacrimal gland, atrophy of the acinar cells in the lacrimal gland, and increased vacuolation in the adrenal zona fasciculata cells were observed in males, and a decrease in spontaneous activity and ataxic gait in 1 female and clonic convulsion in another female (satellite group) were observed on 1 day.

At 300 mg/kg (males) and 200 mg/kg (females), 1 male and 4 females died within 2 hours of dosing on Day 2, 17, 39, 65, or 86 of dosing, and clonic convulsion was observed in these animals before death. For the animals that died and survived, clonic convulsion, ataxic gait, abnormal position (prone or lateral position), a decrease in spontaneous activity, salivation, and high total cholesterol were observed in both sexes. In males, a decrease in stool volume, emaciation, and trace of reddish rhinorrhea in 1 male and mydriasis in another male were observed, and low body weight, low food consumption, low triglycerides, high calcium and inorganic phosphorus, dark discoloration of the lacrimal gland with atrophy of the acinar cells in the lacrimal gland, and increased vacuolation in the adrenal zona fasciculata cells were observed. In females, high adrenal and kidney weights were observed.

Other than those stated above, the following changes considered to be related to drug metabolizing enzyme induction were observed: high liver weight in males and females at 100 mg/kg and more, and hypertrophy of the centrilobular hepatocytes in males at 300 mg/kg and females at 200 mg/kg.

The changes noted during the dosing period recovered or tended to recover during the 4-week recovery period.

In toxicokinetics, Cmax increased dose-dependently in both sexes on Days 1, 49, and 91 of dosing, except at 100 to 300 mg/kg for males and at 30 to 100 mg/kg for females on Day 49 of dosing. There was no difference in Cmax between 100 and 300 mg/kg for males or between 30 and 100 mg/kg for females on Day 49 of dosing. AUC24 generally increased more than dose-proportionally in both sexes on Days 1, 49, and 91 of dosing. Cmax and AUC24 between the same dose levels and between the highest dose levels (males 300 mg/kg and females

200 mg/kg) were higher in females than in males on Days 1, 49, and 91 of dosing, except the Cmax at 100 mg/kg on Day 49 of dosing (comparable in both sexes). Cmax and AUC24 were almost constant in the lowest and the second lowest dose groups and slightly decreased in the other dose groups after repeated dosing. Plasma concentrations of PROJECT S just after death in the male and females in the highest dose groups that died after showing convulsion were　approximately 2 to 4 times higher than Cmax values in corresponding dose groups during the dosing period.

It was concluded that, under the conditions of this study, the NOAEL for PROJECT S was 10 mg/kg/day for males and 30 mg/kg/day for females because low triglycerides were

observed in males at 30 mg/kg and convulsion, a decrease in spontaneous activity, and ataxic gait were each observed once in 1 female at 100 mg/kg. The changes noted during the dosing period were reversible.

### Repeat-dose Toxicity in Dogs

### Exploratory 1-week Oral Dose Range Finding Studies

In the 1-week oral dose range-finding study in dogs (Study Project S-TX-0016; EOTT 3.1), vomiting was observed from the lowest dose of 5 mg/kg onward. Salivation, ataxic gait, decreased locomotor activity, tonic convulsion, prone position, lateral position, increased heart rate and elevated ALT were found at ≥ 30 mg/kg. At 30 mg/kg, tremor, decreased food consumption and increased white blood cells (neutrophil) were also detected. The female at 100 mg/kg was sacrificed in extremis at approximately 1 hour after dosing on day 1 and showed loss of locomotor activity, inability to stand, bradypnea, increases in reticulocyte, AST and glucose and decreases in K and Cl. Cmax and AUC24 values on days 1 and 7 increased in a dose-dependent manner in most animals. There were no apparent differences between 5 and 10 mg/kg doses in males. Cmax and AUC24 values increased slightly after repeat dosing; no gender differences were observed in these parameters.

In the 1-week oral dose range-finding study at 0.5 and 2 mg/kg in dogs (Study Project S-TX- 0017; EOTT 3.1), plasma drug concentrations after single and 1-week dosing regimens reached Cmax rapidly and increased with increasing dose. No differences were observed between single and repeated dosing and no clear differences between the sexes in plasma unchanged drug concentrations were observed. No treatment-related vomiting was observed up to 2 mg/kg.

### Definitive 4-week Oral Toxicity Study

In the 4-week repeat dose oral toxicity study in dogs (Study Project S-TX-0006; EOTTs 3.1, 3.3,

3.4 and 3.7.2), at 30 mg/kg, convulsion was observed on days 16 and 26 of dosing in a female. In the female, staggering gait (on days 1, 2, 3, 8 and 28), decreased locomotor activity (on days 15, 16, 21 and 26), vomiting (21 days/4 weeks), salivation

(25 days/4 weeks), lower body weight and changes in electrocardiogram (ECG) (prolongations of QT and QTc intervals and an extension of QRS, at pre-dosing and/or 1 hour after dosing in week 4) were also noted. In the other animals, the following signs were observed within 1 hour after dosing on each day: staggering gait in 2 males and 1 female

(1 day/4 weeks), decreased locomotor activity in 1 male and 1 female (1 day/4 weeks), inability to stand in 1 male and 1 female (1 day/4 weeks), vomiting in all males and 5 females (5 to 23 days/4 weeks) and salivation in 4 males and 3 females (1 to 25 days/4 weeks) were noted. Relative liver weights were increased in 1 male and 1 female. All findings resolved after drug withdrawal. Cmax and AUC24 values increased more than dose-proportionally at all　sampling points in both sexes, except the 3 mg/kg group in which values increased almost dose-proportionally. There was no appreciable difference between sexes. Toxicokinetic parameters were almost constant regardless of the frequency of administration. The NOAEL was 10 mg/kg/day.

### 4.6.2.2.3 Definitive 13-week Oral Toxicity Study

In the 13-week repeat dose oral toxicity study in dogs [Project S-TX-0022; EOTTs 3.1, 3.3, 3.4 and 3.7.4] no test article-related changes were observed at dose levels of 3 or 10 mg/kg.

At 30 mg/kg, tonic convulsion was noted in 1 female on 7 occasions from Days 35 to 91 at about 20 or 25 minutes after dosing; each convulsion continued for 1 to 3 minutes. A convulsion was also noted once in another female about 20 minutes after dosing on Day 26 of dosing and the convulsion continued for 4 minutes. In addition, ananastasia in 1 male and

4 females, decrease in locomotor activity in 2 males and 3 females, and staggering gait in 3 males and 4 females were noted within 1 hour after dosing. Vomiting in 5 males and

6 females and salivation in 2 males and 3 females were also noted within 1 hour after dosing. Abnormal stools (soft or mucous stool) were sporadically noted in 1 male and 1 female during the dosing period.

The findings observed in the dosing period were not observed during the recovery period.

In the toxicokinetics, the Cmax and AUC24 values increased more than dose-proportionally over the dose range of 3 to 30 mg/kg. These values were almost constant after repeated dosing. There were no appreciable sex differences. Plasma samples were collected immediately after the end of each convulsion, and concentrations of the compound were measured. Those plasma concentrations were approximately 1- to 3-times higher than the mean Cmax values in females at 30 mg/kg. Moreover, these plasma concentrations were generally > 2400 ng/mL, which is defined as the NOEC for convulsions (see Sectio[n 4.6.7.2](#_bookmark68)). One dog showed multiple convulsions, and the plasma concentration following the last convulsion was 1719 ng/mL. The lower plasma concentration suggests that repeat convulsions in this dog increased the sensitivity for next convulsions, which was considered not to impact the overall NOEC for convulsions.

From these results, it was concluded that the NOAEL under the conditions of this study was 10 mg/kg/day. The changes noted during the dosing period were reversible.

### Genotoxicity

The potential genotoxicity of PROJECT S was evaluated in studies in vitro and in vivo with PROJECT S.

### Reverse Mutation Test

To assess the potential of Project S to induce gene mutation, an in vivo bacterial reverse mutation test was performed (Study Project S-TX-0007; EOTTs 3.1, and 3.8) with

5 strains of bacteria [*Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (WP2*uvrA*)] with and without metabolic activation. Compared with the negative control, a 2-fold or greater increase in the number of revertant colonies was not　observed in any test strain in the dose-finding test or the main test. It was concluded that Project S did not induce gene mutation in bacteria.

### Chromosomal Aberration Test

A study was performed in vitro (Study Project S-TX-0008; EOTT 3.1, and 3.9) to assess the potential of Project S to induce chromosomal aberrations in Chinese hamster lung (CHL/IU) cells with short-term treatment with and without metabolic activation and with continuous treatment without metabolic activation. A statistically significant increase

(p < 0.05) in the incidence of cells having structural chromosomal aberrations in short-term treatment without metabolic activation was noted; however, the increase was not particularly high (7.5%) and was observed only at 194 µg/mL, the highest observational dose, associated with 57.7% cytotoxicity. A statistically significant increase (p < 0.05) in the incidence of cells having numerical chromosomal aberrations in continuous treatment for 24 hours was noted; however, the increase was low (4.0%) and observed only at 30.1 µg/mL. However, the potential to induce structural and numerical chromosomal aberrations was weak.

### Micronucleus Test

A micronucleus study was conducted in vivo (Study Project S-TX-0009; EOTT 3.1, 3.3, 3.4, and 3.10) in ICR strain male and female mice (Crlj:CD1(ICR)) to examine the clastogenic potential of Project S. There were no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes in any PROJECT S administration group compared with the control group. In the incidence of polychromatic erythrocytes in total, there were no statistically significant decreases in the 31.25, 62.5 and 125 mg/kg/day (maximum non-lethal dose) groups compared with the control group. It was determined that Project S had no clastogenic potential in bone marrow cells in mice of either gender. In the toxicokinetic analysis at 125 mg/kg/day, the Cmax and AUC24 also showed no marked gender differences. In a dose-range-finding phase, at 500, 1000 and 2000 mg/kg/day, all males and females were found dead; one-third of the males and one-third of the females in the 250 mg/kg/day group were found dead. Decreased spontaneous movement, hypothermia and tremor had been observed in females in the 250 mg/kg/day group.

### Unscheduled DNA Synthesis Test

In an in vivo/in vitro Unscheduled DNA Synthesis (UDS) test (Study Project S-TX-0010; EOTT 3.1, 3.3, 3.4, and 3.11), Project S was evaluated for the ability to induce UDS in male rat hepatocytes in order to evaluate the ability to induce DNA damage in vivo. The mean number of net grains and the percentages of cells in repair obtained from animals treated with PROJECT S at 500 and 1000 mg/kg were < 5 and < 20%, respectively, and no mortality was observed at these doses. It was concluded that PROJECT S showed no potential to induce DNA damage in vivo.

At 250 mg/kg, 1 animal was in a prone position, had convulsion, salivation, then died between 0 and 1 hour after administration. Two of 3 animals in the 1000 mg/kg group were found in a lateral position, had clonic convulsion, irregular respiration and salivation; additionally, 1 showed a reddish tear at that time. Some other treated animals in the 2-hour

and 16-hour post-treatment groups showed decreased locomotor activity and/or ataxic gait between 0 and 1 hour after administration.

### Carcinogenicity

Long-term carcinogenicity studies have not been conducted with PROJECT S.

### Reproductive and Developmental Toxicity

A study to assess the effects of PROJECT S on male and female fertility and early embryonic development to implantation was conducted in rats [Project S-TX-0023]. Studies to assess the effects of PROJECT S on embryo-fetal development were conducted in rats and rabbits [Project S- TX-0011, Project S-TX-0012, Project S-TX-0013, Project S-TX-0014].

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

To examine the toxicological effects on fertility of male and female rats and early embryonic development to implantation, PROJECT S was administered from 4 weeks before the commencement of mating until the day before gross pathological examination for males, and from 2 weeks before the commencement of mating through Day 7 of gestation for females [Project S-TX-0023; EOTT 3.1, 3.3, 3.4, and 3.17].

No test-article related changes were observed in male and female rats at 10 or 30 mg/kg.

In the 100 mg/kg group, lateral position with generalized weakness was observed in one male on Day 15 of dosing, and one female died on Day 22 of dosing. Additional findings observed for females in this group were as follows: clonic convulsion, lateral position, prone position, staggering gait, tremor, decreased spontaneous motility, and salivation. No test article-related abnormalities in general signs were observed at 10 or 30 mg/kg in males and females. One male in the 30 mg/kg toxicokinetic-satellite group was found dead before dosing on Day 13 of dosing. Since all deaths in rats (except for one after treatment at 1000 mg/kg in the 4-week toxicity study) were preceded by convulsions, and convulsions are extremely unlikely to occur at the plasma exposures reached at 30 mg/kg, the death was considered not related to treatment with PROJECT S. No test article-related effects on body weight or food consumption were noted. No test article-related effects on the estrous cycle, numbers of corpora lutea, copulation index, fertility index, or days until copulation were noted in males or females. No test article-related abnormalities were observed on gross pathology at any dosages in either sex.

At necropsy on Day 14 of gestation, no statistical significant differences in the number of implantations, live embryos, or embryo-fetal deaths, pre- or postimplantation loss rate were observed between the treated and control groups.

The tmax values were 0.5 h on Days 1 (both sexes), 14 (females), and 28 (males) of dosing in all dosing groups except for the 100 mg/kg group in females on Day 1 (1 h). The Cmax values in males increased more than dose-proportionally on Day 1 but almost dose proportionally on Day 28. AUC24 values in males increased more than dose-proportionally on both Days 1 and

28. Values of Cmax and AUC24 on Day 28 were low compared with Day 1, except for the 10 mg/kg group. Those in the 10 mg/kg group on Day 28 were high compared with those on

Day 1. Cmax values in females increased dose-proportionally on Day 1 and less than dose- proportionally on Day 14. AUC24 values in females increased more than dose-proportionally on Day 1 and almost dose proportionally on Day 14. Values of Cmax and AUC24 on Day 14 were low compared with those on Day 1, except for Cmax in the 10 mg/kg group, which were almost the same as those on Day 14. PROJECT S was not detected in plasma of the control group for either sex. On Day 1, Cmax and AUC24 values in females were higher than those in males in all groups.

In summary, in the 100 mg/kg group, clinical signs were noted in one male and most females and one female died. From these results, it was concluded that, under the conditions of this study, the NOAEL for parental general toxicity was 30 mg/kg/day for males and females.

The NOAELs for male and female reproductive function and early embryonic development were judged to be 100 mg/kg/day.

### Effects on Embryo-fetal Development

### Exploratory Study in Rats

An exploratory study was performed to investigate the effects on dams and embryo-fetal development and to set the dose levels for the subsequent main study of the effects on embryo-fetal development in rats (Study Project S-TX-0011; EOTTs 3.1, and 3.13). At

100 mg/kg, mydriasis was found in pregnant rats. No test article-related abnormalities were observed in body weight, body weight gain, food consumption or gross pathological findings in any group. No study drug-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 or placental findings in any group. It was determined that

300 mg/kg would be appropriate for the high-dose level in the subsequent main study of the effects on embryo-fetal development in rats.

### Definitive Study in Rats

A definitive study of embryo-fetal development was performed in rats (Study Project S-TX-0012; EOTTs 3.1, 3.3, 3.4, and 3.14) to investigate the effects of PROJECT S on pregnant females and embryo-fetal development and to assess systemic exposure to PROJECT S. No effects were seen at 30 mg/kg and minimal suppression of body weight gain was seen during the dosing period in the 100 mg/kg group. In the 300 mg/kg group, no dams died in the toxicity group; however, 1 dam in the satellite group died after first dosing. In this group, a decrease in spontaneous activity, prone position and ataxic gait were observed. Loss of fur and dirty lower belly by urine were also observed during the late gestation period. Body weights on days 12 to 20 of gestation and food consumption during the dosing period (days 7-17) were significantly lower than those in the control group and body weight gains were significantly suppressed throughout the dosing period. No PROJECT S-related changes were observed in the number of corpora lutea or implantations in any PROJECT S group. In the 300 mg/kg group, low fetal and placental weights, significant increases in external and skeletal abnormalities, visceral variations and delayed skeletal ossifications in sternebrae, sacral and caudal vertebrae, metacarpi and metatarsi were observed. The frequency of external abnormalities

in the 300 mg/kg group was 78.8%; the main phenotypes for these were local edema (64.7%), cleft palate (61.0%), mandibular micrognathia (52.0%) and adhesion of the maxilla and tongue (17.5%). Among skeletal abnormalities, in addition to findings associated with cleft palate and mandibular micrognathia, misshapen scapula (52.7%), unossified vertebral centrum (37.2%) and kyphosis of vertebral column (25.3%) were observed with high frequencies. As a visceral variation, the frequency of thymic remnant in the neck (17.2%) was significantly higher than in the control group. In the 300 mg/kg group, no test article- related changes were noted in the number of live fetuses, number of embryo-fetal deaths, postimplantation loss rate, frequency of placental or visceral abnormalities or frequency of skeletal variations.

Cmax values on days 7 and 17 of gestation increased less than dose-proportionally up to 300 mg/kg, except at the 100 mg/kg dose on day 7; at that point, the Cmax value increased more than dose-proportionally. AUC24 values in both stages increased more than

dose-proportionally up to 300 mg/kg. In the 300 mg/kg group, tmax values were delayed in both stages.

It was determined that the NOAEL of PROJECT S was 30 mg/kg/day for dams (general toxicity) and 100 mg/kg/day for embryo fetal development.

### Exploratory Study in Rabbits

An exploratory study was performed in rabbits (Study Project S-TX-0013; EOTTs 3.1, 3.3, 3.4 and 3.15) to examine the general toxicological effects of PROJECT S on nonpregnants, then on dams and on embryo-fetal development. Doses used were 30, 100 or 300 mg/kg/day in the preliminary trial using nonpregnants and the animals were orally treated for 1 or 5 days. At 300 mg/kg/day, animals were in a lateral position and exhibited opisthotonus and clonic convulsion immediately after the first dosing then died; the remaining survivor was found in a lateral position transiently and was sacrificed on day 1 of administration. Treatment at 100 mg/kg/day for 5 days induced clinical signs of CNS involvement including lateral position, opisthotonus, clonic convulsion, staggering gait and salivation, as well as slightly decreased body weight and food consumption. At 30 mg/kg/day, there were no changes in general condition. On days 1 and 5, Cmax and AUC24 values increased more than dose-

proportionally up to 100 mg/kg/day, except for Cmax values on day 5, which increased almost dose-proportionally. Cmax and AUC24 values did not change with dosing time. In the preliminary study with pregnant rabbits, treatment at 100 mg/kg/day induced slightly decreased fecal output, body weight and food consumption, as well as transient staggering gait and one abortion. At ≤ 30 mg/kg/day, no treatment-related findings were observed. No treatment-related effects, such as embryo-fetal death or change in fetal body weight, placental weight and sex ratio, including external and macroscopic abnormalities in the fetuses and placentae were observed at any dose employed. In addition, no significant increases were seen in visceral abnormalities and variations at any dose. In summary, no treatment-related effects such as fetal lethality, inhibition of fetal growth or teratogenicity were observed at any dose employed.

### Definitive Study in Rabbits

A definitive study of embryo-fetal development was performed in pregnant rabbits

(Study Project S-TX-0014; EOTTs 3.1, 3.3, 3.4, and 3.16) to investigate the effects of PROJECT S on pregnant females and on embryo-fetal development at oral doses of 0, 10, 30 and 100 mg/kg and to assess systemic exposure to PROJECT S. Treatment at 100 mg/kg/day induced CNS involvement including lateral position, clonic convulsion and/or opisthotonus, slightly decreased body weight and food consumption and death. At ≤ 30 mg/kg/day, there were no treatment-related findings. There were no significant differences in the number of corpora lutea or implantations between the control group and any PROJECT S group. Suppression of fetal growth, as expressed by reduced fetal body weight and reduced skeletal ossification of sacral and caudal vertebrae, was observed at 100 mg/kg/day but not at ≤ 30 mg/kg/day. No treatment-related effects, such as embryo-fetal death or changes in placental weight or sex ratio including external and macroscopic abnormalities in the fetuses and placentae, were observed at any dose employed. In addition, no significant increases were seen in visceral or skeletal abnormalities or variations, skeletal abnormalities or variations, including placental abnormalities, in any treated group. In summary, no treatment-related findings were recorded on embryo-fetal development, such as fetal lethality and teratogenicity, under these conditions at any doses employed, although treatment at 100 mg/kg/day induced inhibition of fetal growth. Cmax and AUC24 values in both stages increased more than dose-proportionally up to 100 mg/kg/day; however, Cmax values on gestation day 18 increased less than dose- proportionally from 30 to 100 mg/kg/day. Toxicokinetic parameters were mostly comparable after the first and final dosing. The NOAEL of PROJECT S was 30 mg/kg/day for general toxicological and reproductive effect in dams and embryo-fetal development.

### Local Tolerance

Local tolerance studies (eye irritation, hemolytic action in human peripheral blood, or venous and perivascular irritancy) with PROJECT S were not performed.

### Other Toxicity Studies

Additional studies were performed in rats and dogs to examine the relationship between occurrence of clinical signs including convulsion and plasma drug concentration [Project S-TX- 0025 and Project S-TX-0020]. These studies were preceded by exploratory studies in rats and dogs to define the escalating dose steps for the continuous infusion and are described in the EOTT only [Project S-TX-0024; EOTT 3.1 and 3.22 and Project S-TX-0019; EOTTs 3.1, and 3.20].

### Definitive Study in Rats by Continuous Intravenous Infusion

In the definite study in rats [Project S-TX-0025; EOTT 3.1 and 3.23] PROJECT S was administered by continuous intravenous infusion to rats. Dosing solution was administered according to the schedule described in [(Table 2](#_bookmark67)).

### Table 2 Administration Manner

|  |  |  |  |
| --- | --- | --- | --- |
| **Phase** | **Infusion Rate (mg/kg/min)** | **Infusion Rate (mL/kg/min)** | **Infusion Duration (min) †** |
| Loading | 0.4 | 0.04 | 30 (30) |
| Infusion 1 | 0.16 | 0.016 | 35 (65) |
| Infusion 2 | 0.52 | 0.052 | 60 (125) |
| Infusion 3 | 1.04 | 0.104 | 60 (185) |

†: Time in the parenthesis shows total time after start of administration.

During the loading and infusion 1 phases, no abnormalities were observed in any rat.

During the infusion 2 phase, a decrease in locomotor activity was observed in 5 males and 6 females. During the infusion 3 phase, a decrease in locomotor activity was observed in

5 males and 6 females, and prone and lateral position were observed in 4 males and 4 females. Convulsion (tonic and/or clonic) was observed in all male and female rats from 128 to

146 minutes after the start of administration. There was no appreciable sex difference in the clinical signs.

The plasma drug concentrations generally increased with the higher infusion rate. There was no appreciable sex difference in plasma drug concentrations. The mean plasma concentrations of PROJECT S at the end of the infusion 1 phase were 4031.60 ng/mL in males and 4202.84 ng/mL in females. The mean plasma drug concentrations at the end of the infusion 2 phase were 13151.03 ng/mL in males and 11931.47 ng/mL in females. The mean plasma drug concentrations immediately after the end of convulsion were 17039.40 ng/mL in males and 16972.27 ng/mL in females. The lowest individual plasma concentration

immediately after the end of convulsion was 9745.53 ng/mL which was defined as the LOEC. The lowest individual plasma concentration at the end of the infusion 2 phase (phase without convulsions in any rat) was 7636.86 ng/mL, which was considered the NOEC.

### Definitive Study in Dogs by Continuous Intravenous Infusion

In a definitive study with continuous intravenous infusion in dogs (Study Project S-TX-0020; EOTTs 3.1 and 3.21), Project S was administered by continuous intravenous infusion to both male and female beagle dogs using an implanted catheter. At the onset of clinical signs, drug concentration in plasma were examined. The dosing solution at

2.5 mg/min PROJECT S was loaded for 5 minutes. The dosing solution was then administered at 1 mg/min PROJECT S for 4 hours; subsequent infusion rate was 2 mg/min PROJECT S for 180 minutes and 3 mg/min PROJECT S for 37 minutes. In dogs showing convulsions during the 2 mg/min infusion period (3 males and all females) and during the 3 mg/min infusion period (1 male), infusion was stopped after blood sampling for toxicokinetic analysis.

Clinical signs occurring prior to the convulsions were limited to vomiting, staggering gait and decreased locomotor activity. Immediately after manifestation of convulsion, the plasma concentration of PROJECT S of those animals ranged from 3179.74 to 4856.29 ng/mL. The lowest individual plasma concentration associated with a convulsion was defined as the LOEC and was about 3200 ng/mL. The plasma exposure at the end of the period with an infusion rate that did not induce convulsions (generally 1 mg/min, with one exception at

2 mg/min) ranged from 2392 to 3905 ng/mL. The NOEC was determined to be 2400 ng/mL.

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

PROJECT S is a state-dependent inhibitor of the N-VDCC, which is the predominant subtype in the DRG neuron and the superficial lamina of the spinal dorsal horn, suggesting they are located presynaptically in afferent C- and Aδ-fiber terminals, where they control the release of neurotransmitters such as glutamate and substance P. Essentially, the function of N- VDCC is a “triggers” for nociceptive signal transmission; PROJECT S inhibits nociceptive signal transmission by blocking N-VDCC. PROJECT S demonstrated efficacy in neuropathic and visceral pain models that was not confounded by effects on locomotor activity or sedation.

In in vitro studies, PROJECT S inhibited N-VDCC in the FLIPR Ca2+ imaging assay and inhibited the inactivated state in this channel approximately 36 times more potently than the resting state in the electrophysiological assay. These data indicate PROJECT S is a state- dependent inhibitor of N-VDCC.

In in vivo studies, PROJECT S exerts a significant analgesic effect in models of neuropathic and visceral pain at a dose range from 0.3 to 3.0 mg/kg in the rat. The effects of PROJECT S at the pharmacologically active dose range were not confounded by toxicity effects. Tolerance or sensitization to the analgesic effect did not occur, as demonstrated in a repeat-dose pharmacology study that examined mechanical allodynia in the SNL model.

Overall, PROJECT S demonstrated activity in animal models sensitive to both neuropathic pain and nociceptive pain. In addition, PROJECT S may be useful as a drug for concomitant administration with morphine for treatment of refractory chronic pain such as cancer pain and chronic postoperative pain.

Following single administration to rats and dogs, PROJECT S had a relatively short t½ (1.0 to

5.3 hours) and low oral bioavailability, which ranged from 2.9% to 9.3%. PROJECT S has a relatively high brain-to-plasma ratio, indicating it penetrates into the brain relatively easily. Furthermore, maximum brain concentrations in rats are reached at the same time as maximum plasma concentrations, suggesting that PROJECT S enters into the brain rapidly without any delay. A correlation study using human liver microsomes indicated that PROJECT S is mainly metabolized by CYP3A4/5, although CYP2B6 and CYP2D6 may also be involved in the metabolism of PROJECT S. In vitro metabolic fingerprinting was conducted using liver microsomes and hepatocytes. Although more than 25 metabolites were observed in human hepatocytes, no human-specific metabolites were detected [[Section 4.6.3](#_bookmark51)].

In toxicology and safety pharmacology studies, the major findings at high doses were CNS clinical signs, including convulsion across species and cardiovascular and respiratory system changes in conjunction with convulsions in dogs [Section[s 4.3,](#_bookmark20) [4.6.1,](#_bookmark39) [4.6.2](#_bookmark42) and [4.6.5](#_bookmark57)].

Plasma potassium and calcium concentrations were reduced in dogs [[Section 4.3](#_bookmark20)].

Total cholesterol was increased in rats and a decrease in triglycerides and shortened clotting time were noted in male rats without evidence of histopathological changes in the liver.

Histopathological changes were observed in adrenal and lacrimal glands of male rats. The

increased vacuolation in the adrenal zona fasciculata was very slight and therefore considered of low toxicological significance. Atrophic findings in the lacrimal gland were not accompanied by changes in the eye or other exocrine gland (submandibular or sublingual gland) and the toxicological significance was unclear. All changes were reversible

[[Section 4.6.2](#_bookmark42)].

PROJECT S did not affect male or female rat fertility, but was shown to be teratogenic in rats at maternal toxic doses [Section [4.6.5](#_bookmark57)]. PROJECT S had no genotoxic potential in vivo

[[Section 4.6.3](#_bookmark51)].

Due to the lack of clear preceding signs for convulsions in the animal studies, an exposure cap is set for clinical studies. Since convulsions were seen in rat and dog following acute dosing, and the incidence did not increase with repeat dosing, the convulsions are regarded to be related to plasma concentration and not AUC. Continuous infusion studies were conducted in rats and dogs to establish the threshold plasma concentration of PROJECT S associated with convulsions. Dogs were found to be the most sensitive species and the NOEC for convulsions was defined at 2400 ng/mL. This method revealed more precisely the plasma concentration associated with convulsions compared to the standard Cmax levels obtained in the 4- and 13-week repeated dose toxicity studies. In the dog 13-week oral dose toxicity study, the plasma concentrations of PROJECT S were also measured following each observation of a convulsion and were typically > 2400 ng/mL.

It was concluded that the NOEC of 2400 ng/mL obtained in the dog by continuous infusion is applicable for exposure cap setting in both single and multiple dose clinical studies.

Exposures obtained during the 4- and 13-week toxicity studies are provided in [[Table 3](#_bookmark70)].

### Table 3 Summary of Exposures Obtained in Repeated Dose Toxicity Studies with PROJECT S

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species/**  **Study Duration** | **Dose** | **Sex (M/F)** | **AUC24**  **(ng·h/mL)** | **Cmax (ng/mL)** | |  |
| Rat/  13-week po | 10 mg/kg (NOAEL) | M | 537 | 312 | |
| 30 mg/kg (NOAEL) | F | 7948 | 4824 | |
| 30 mg/kg (LOAEL) | M | 2899 | 1183 | |
| 100 mg/kg (LOAEL) | F | 22500 | 7116 | |
| Rat Embryo-fetal  developmental toxicity study | 100 mg/kg (NOAEL for  teratogenicity) | F | 22434 | 3759 | |
| 300 mg/kg (LOAEL for  teratogenicity) | F | 104413 | 9464 | |
| Dog/  13-week po | 10 mg/kg (NOAEL) | M | 362 | 165 | |
| F | 263 | 142 | |
| 30 mg/kg (LOAEL) | M | 2559 | 996 |  |  |
| F | 2963 | 1483 |  |
| Highest dose from SAD  [Project S-CL-0001] | 50 mg |  | 733(AUCinf) | 78 | |  |

AUC24: area under the concentration-time curve, from 0 to 24 hours after dosing; Cmax: maximum plasma concentration; po: by mouth; NOAEL: no-observed –adverse-effect level; LOAEL: lowest-observed adverse-effect level.

A summary of potential safety concerns for clinical trials, as well as recommended risk minimization actions is provided in [Table 12](#_bookmark112) (Section [6.4](#_bookmark111)).