### Safety Pharmacology

A tabulated overview of safety pharmacology studies may be found in End-of-Text Table 1.2.

Results of the nonclinical safety pharmacology studies indicated:

* + - * PROJECT Q does not affect the hERG current up to 3 mcM but showed a suppression effect on the hERG current at the highest test concentration of 30 mcM; its compensated suppression rate was 22%.
      * PROJECT Q does not affect the action potentials in isolated guinea pig papillary muscles at a concentration of up to 10 mcM.
      * In dogs, PROJECT Q has no effect on the cardiovascular or respiratory system at the dose of 15 mg/kg. At the dose of 60 mg/kg, vomiting, vomitus eating, retching, scratching, a decrease in body temperature and an increase in serum potassium were

observed. In addition, at doses of 600 and 1500 mg/kg, staggering, retching, twitch, salivation, compound-colored feces, incontinence of urine, drowsiness, tremor, a prolongation of QTc interval (as corrected by Fridericia’s formula), a decrease in pH, and an increase in arterial blood PaCO2, an increase in serum sodium and chloride level was observed [End-of-Text Table 1.4].

* + - * In rats, PROJECT Q did not affect the body temperature, general activity and behavior up to 24 h after administration at doses of 30, 300 or 2000 mg/kg. PROJECT Q has no effect on the CNS at doses of up to 2000 mg/kg [End-of-Text Table 1.3]

### Pharmacodynamic Drug Interactions

Nonclinical pharmacodynamic drug interaction studies have not been conducted.

## Toxicology

The current toxicological data package for PROJECT Q includes 4 pivotal safety pharmacology studies, 2 pivotal single oral dose toxicity studies, 3 nonpivotal 1-week oral dose toxicity studies, 2 pivotal 4-week oral dose toxicity studies with recovery assessment, 2 pivotal genotoxicity studies, 2 nonpivotal studies of effects on embryo-fetal development, 2 pivotal studies of effects on embryo-fetal development and 2 pivotal immunotoxicity studies.

Pivotal studies were conducted under appropriate International Conference on Harmonisation (ICH) guidelines/guidances and in accordance with GLP standards.

A tabulated overview of these studies may be found in End-of-Text Table 3.1.

### Single-dose Toxicity

A tabulated overview of single-dose toxicity studies may be found in End-of-Text Table 3.5.

### 4.3.1.1 Rats (Project Q-TX-0004)

In a study in rats (Project Q-TX-0004), PROJECT Q was singly administered orally at dose levels of 1000 and 2000 mg/kg to 5 male and 5 female rats per group in order to investigate its toxicity. No animal died in any dose group. There were no test-article related changes in clinical signs, body weight, body weight gain or gross pathology in any dose group. The approximate lethal dose (LD) in rats was greater than 2000 mg/kg for males and females [End-of-Text Table 3.5.1].

### 4.3.1.2 Dogs (Project Q-TX-0005)

In a study of dogs (Project Q-TX-0005), PROJECT Q was single administered orally at dose levels of 750 and 1500 mg/kg to 1 male and 1 female dog per group in order to investigate its toxicity. Systemic exposure to PROJECT Q was also assessed. No animal died or was euthanized due to moribundity and no test-article related changes were observed in gross pathology or histopathology at either dose level.

At 750 mg/kg, vomiting was observed in the male dog approximately 2 h after dosing and in the morning on day 1, and in the female dog approximately 3 and 5 h after dosing. In the female, decrease in food consumption was observed on day 0 only; body temperature decreased 4 h after dosing and recovered by 24 h after dosing. Increases in blood urea nitrogen (BUN) and sodium (Na) in both male and female, increases in creatinine and inorganic phosphorus (IP) in the male and an increase in Cl in the female were observed on day 1, and all of these changes were recovered by day 7.

At 1500 mg/kg, vomiting was observed in the male dog approximately 2 and 3 h after dosing and in the female dog approximately 4 h after dosing. Additionally, in the male, a decrease in spontaneous activity and incomplete eyelid opening were observed from approximately

5 to 6 h and 6 h after dosing, respectively, subsequently recovered by the morning on day 1. The male showed no food consumption on day 1 and decrease in body weight on day 2, both

of which were recovered on day 2 and day 3, respectively. Body temperature decreased in the male and female 4 to 24 h and 4 h after dosing, respectively, and was recovered 48 h and 24 h after dosing, respectively. Increases in BUN, Na and Cl in both the male and female, increases in creatinine and IP in the female, and a decrease in lymphocyte count in the male were observed on day 1 and all of these changes were recovered by day 7.

In toxicokinetics, Cmax values at 750 and 1500 mg/kg were 29,555.87 and 43,904.71 ng/mL in males and 28,595.79 and 17,981.81 ng/mL in females, respectively. AUC24 values at the corresponding doses were 529,466.67 and 889,101.37 ng·h/mL in males and 517,194.51 and 298,493.85 ng·h/mL in females, respectively. Tmax was 8 h in both sexes at both dose levels. Cmax and AUC24 increased in a dose-related manner in males, no apparent sex difference was noted at 750 mg/kg, while Cmax and AUC24 at the dose of 1500 mg/kg were lower than those at 750 mg/kg in females. The approximate LD in dogs was greater than 1500 mg/kg for males and females [End-of-Text Table 3.5.2].

### Repeated-dose Toxicity

Nonpivotal dose range-finding and pivotal oral repeated dose studies of PROJECT Q were conducted in rats and dogs. A tabulated overview of repeat-dose toxicity studies may be found in End-of-Text Tables 3.6 and 3.7.

### Rat

### Nonpivotal 7-day Oral Toxicity (Project Q-TX-0001)

PROJECT Q (amorphous formulation) was administered orally to 4 male and 4 female rats once daily for 7 days at doses of 0 (vehicle: 3%, TC-5, R solution), 30, 100, and 300 mg/kg/day (Project Q-TX-0001). Systemic exposure to PROJECT Q was also investigated.

No animals died during the dosing period. No test-article related changes were noted in any dose group in clinical signs, body weight, food consumption, hematology, blood chemistry, gross pathology and organ weight.

In histopathology, narrowing of the splenic marginal zone was observed at 100 mg/kg or more in males and at 300 mg/kg in females.

In toxicokinetics, Cmax and AUC24 in both sexes increased dose-dependently at the first and final days of dosing except in females in the 300 mg/kg/day group at final day of dosing.

Cmax and AUC24 were higher in females than that in males at the first and final days of dosing. Tmax was comparable in males and females at the same dosage and did not change by repeated administration. Plasma concentrations of PROJECT Q did not change following 7-day repeated dosing. The NOAEL was 30 mg/kg/day for males and 100 mg/kg/day for females [End-of-Text Table 3.6.1].

### Nonpivotal 7-day Oral Toxicity, Additional High Dose (Project Q-TX-0002)

PROJECT Q (spray-dried amorphous formulation) was administered orally to 5 male and

5 female rats once daily for 7 days at doses of 0 (vehicle: 5% TC-5, R solution), 100, 300 and 1000 mg/kg/day (Project Q-TX-0002). Systemic exposure to PROJECT Q was also investigated.

No animal died in any dose group during the dosing period. No test article-related changes were noted in any group in clinical signs, body weight, hematology, gross pathology and organ weight.

At 100 mg/kg, histopathology of the glandular stomach revealed atrophy of the surface epithelium in 1 male and hemorrhage in the lamina propria in 1 female. At 300 mg/kg/day or more, histopathology revealed narrowing of the marginal zone in the spleen and atrophy of the surface epithelium and hemorrhage in the lamina propria in the glandular stomach in male and female rats. These findings in the stomach were considered to be associated with local irritation induced by the dosing formulation. At 1000 mg/kg/day, low food consumption was noted in females on day 1 to 4, but no significant change in body weight was noted. A high calcium concentration was noted in males in blood chemistry.

In toxicokinetics, Cmax and AUC24 increased dose-dependently at the first and final days of dosing in rats, except in males AUC24 on day 1. Cmax and AUC24 in females were higher than those in males in all groups. Tmax was 1.00 to 4.00 h after dosing at the first and final days of dosing and they tended to be longer in females than those in males in the 300 and

1000 mg/kg/day groups. Cmax and AUC24 in males and females on the final day of dosing tended to be lowered than on the first day of dosing at all dose levels, except for males in the 300 mg/kg/day group. The Cmax and AUC24 values were 1.1 to 13.4 times higher in this study (using spray-dried amorphous formulation suspended in 5% TC-5,R solution) than those of the previous study (Project Q-TX-0001: using amorphous form suspended in 3% TC-5, R solution). The NOAEL of PROJECT Q in rats treated with higher doses of test article was less than 100 mg/kg/day for males and females [End-of-Text Table 3.6.2].

### Pivotal 4-week Oral Toxicity (Project Q-TX-0006)

PROJECT Q (spray-dried amorphous formulation) was orally administered once daily for

4 weeks at dose levels of 0 (vehicle: 5% TC-5, R solution), 10, 30, 300 and 2000 mg/kg/day to 10 male and 10 female rats per group in order to investigate its toxicity (Project Q-TX-0006). 5 male and 5 females were added to the control and highest dose groups to assess the reversibility of toxicity during a subsequent 4-week recovery period. A satellite group

(3 males and 3 females in the control group and 6 males and 6 females in each test article group) was added to each dose level to assess systemic exposure to PROJECT Q.

During the dosing and recovery periods, no animal died and no toxic changes were noted in clinical signs, ophthalmology, gross pathology or organ weights at any dose level. At 10 and 30 mg/kg, no test article-related changes were noted.

At 300 mg/kg, slightly low food consumption in males during the dosing period, low albumin in females and narrowing of the splenic marginal zone in both males and females were noted at the end of the dosing period.

At 2000 mg/kg, low body weight gain and food consumption during the dosing period, low body temperature on day 1 of dosing (approximately 2 h after dosing) and high urine volume and low urinary specific gravity at week 4 of dosing were noted in both males and females. At the end of the dosing period, prolongation of PT and APTT in males, low albumin in both males and females, high BUN and low albumin ratio and albumin/globulin ratio in males

were noted. In pathological examinations, narrowing of the splenic marginal zone, which is due to a decrease in the number of B cells in this area indicated by narrowing of the CD45RA positive stain, and focal dilatation of the renal tubule accompanied by basophilic change were noted in both males and females. No toxic changes were noted at 2000 mg/kg during or at the end of the 4-week recovery period.

In toxicokinetics, during the dosing period, Cmax and AUC24 values increased almost

dose-proportionally up to 30 mg/kg and increased less than dose-proportionally from 300 to 2000 mg/kg in both sexes. Those values were almost constant following repeated dosing.

Cmax and AUC24 values in female rats were higher than those in male rates. Tmax values were

0.5 to 4 h and almost constant for the dose range of 10 to 2000 mg/kg in both sexes during the dosing period. The NOAEL was 30 mg/kg/day for male and female rats. Changes noted during the dosing period recovered after 4-week withdrawal [End-of-Text Table 3.7.1].

### Dogs

### Nonpivotal 7-day Oral Toxicity (Project Q-TX-0003)

PROJECT Q (spray-dried amorphous formulation) was administered orally to 1 male and 1 female beagle dog per group once daily for 7 days at doses of 0 (vehicle: 5% TC-5,R

solution), 6, 20, 60 and 600 mg/kg/day. Systemic exposure to PROJECT Q was also evaluated (Project Q-TX-0003).

No animal died or was sacrificed due to moribundity in any dose group. No test article-related changes were noted in any group in food consumption, ophthalmology,

electrocardiography, hematology, gross pathology and organ weight, nor were noted in any observation or examination up to 60 mg/kg group.

At 600 mg/kg, abnormal stool color (yellowish white; this change was considered to be possibly due to the excretion of unabsorbed test article), vomiting (at 1 or 4 h after dosing), a decrease in body weight and cortex atrophy of the thymus in histopathology were noted in the male and female. Degeneration of germinal epithelium in the testes in histopathologic examination was noted in the male. Increases in ALP, BUN, and creatinine in blood chemistry were noted in the female.

In toxicokinetics, tmax ranged from 1.00 to 8.00 h in male and female dogs. Cmax and AUC24 on days 1 and 7 increased in a dose-related manner. No apparent sex differences were noted. Cmax and AUC24 on day 7 were higher than those on day 1 (1.2 to 6.1 times higher for Cmax and 1.2 to 5.9 times higher for AUC24). The NOAEL was 60 mg/kg/day for both male and female dogs [End-of-Text Table 3.6.3].

### Pivotal 4-week Oral Toxicity (Project Q-TX-0007)

PROJECT Q (spray-dried amorphous formulation) was administered orally once daily for

4 weeks at dose levels of 0 (vehicle: 5% TC-5, R solution), 20, 100 and 600 mg/kg/day to 4 male and 4 female beagle dogs per group in order to investigate its potential toxicity (Project Q-TX-0007). 3 males and 3 females were added to the 600 mg/kg group in order to assess the reversibility of toxicity during a subsequent 4-week recovery period. Because

changes in clinical signs (decrease in spontaneous activity, incomplete eyelid opening and/or

somnolence) and decreases in food consumption and body weight were observed in some males in the 600 mg/kg group, the high dose level was decreased to 300 mg/kg for males from day 9 of dosing. These changes were relatively mild in females; therefore, the high dose level was sustained until the last dose. Systemic exposure to PROJECT Q was also assessed.

At 600 mg/kg, 1 female showed lateral position and abnormal stool color (dark red) before dosing on day 28 of dosing and it was euthanized due to moribundity. In this animal, leukocyte and neutrophil counts increased and PT and APTT prolonged in hematology on day 28 of dosing and globulin, glucose, calcium (Ca) and potassium (K) increased in blood chemistry on day 28 of dosing. In histopathology, atrophy of acinar cell in the submandibular gland, decrease in glycogen in hepatocyte and erosions in the ileum, colon and rectum were observed. Other than that, this animal showed similar changes to the other females of this group, including body weight, food consumption, clinical signs, body temperature, urinalysis, electrocardiography, and organ weight.

At the highest dose (male: 600/300 mg/kg or female: 600 mg/kg), marked decreases in food consumption were observed and body weight decreased in both sexes. However, in males after reduction of the dose level to 300 mg/kg (day 9 of dosing), food consumption and body weight recovered, except in 1 male, in which body weight at the end of the dosing period was 78% of the pre-dose value. In females in which body weight decreased, the body weights at the end of the dosing period were 63% to 81% of the pre-dose values. Incomplete eyelid opening and a decrease in spontaneous activity were observed during the dosing period in both sexes. Somnolence was observed in both sexes mainly at weeks 2 and/or 3 of dosing.

Salivation was observed in females from day 14 to 19 of dosing. Vomiting was observed in both sexes sporadically during the dosing period. Scratching and trauma was observed in males. Body temperature decreased in both sexes on days 1 and 8 of dosing and in 1 female in 24 h after dosing on day 26 of dosing [End-of-Text Table 3.7.2].

At the highest dose, prolongation of QRS duration and QTc (as corrected by Matsunaga’s formula) was noted in females on day 26 of dosing. In urinalysis, occult blood was observed, specific gravity decreased and unclassifiable crystals in urinary sediments were observed in both sexes. Total excretions of Na, Cl and/or K decreased in females. In hematology, erythrocyte count, hematocrit value and hemoglobin concentration decreased in both sexes, while these parameters increased in 2 females on day 28 of dosing. In both sexes, platelet count decreased, reticulocyte ratio increased, eosinophil count increased and lymphocyte count decreased on days 15 and/or 28 of dosing. In blood chemistry, in both sexes, AST, ALT, ALP, BUN, creatinine and total cholesterol increased on days 15 and/or 28 of dosing. In females, total bilirubin, total protein, albumin and/or IP increased and Na and/or Cl decreased on days 15 and/or 28 of dosing.

At the highest dose, in gross pathology, multiple white foci in the left kidney were observed in 1 female. In organ weights, high kidney weights in females (including the female euthanized due to moribundity) and 1 male, high lung weights in females and low testis weight in 1 male were observed. High adrenal weights were also observed in females. In histopathology, crystal deposition was observed in giant cell in the lymphoid tissues

(submandibular and mesenteric lymph nodes and Peyer’s patch), lung, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, prostate, and/or skin, in Kupffer cell in the liver, and/or in renal papilla/ureter and renal tubule of the kidney in both sexes. In the kidney, focal dilatation of the renal tubule with mononuclear cell infiltration and/or neutrophil infiltration, focal fibrosis, foreign body granuloma, hemorrhage, hypertrophy in the transitional epithelium and/or regeneration of renal tubule were observed in both sexes. Atrophy and degeneration of the seminiferous tubule in the testis with absence of sperm or cell debris in the epididymis and immaturity in the prostate were observed in 1 male.

Atrophy of the thymus was observed in both sexes. Hyperplasia of the bile duct and atrophy of the pancreatic acinar cell were observed in females. In order to explore the nature of the crystals, an investigative laser microdissection technique was conducted to collect tissue crystal from the lymph node and kidney followed by chemical (LC-MS) analysis.

Preliminary data suggest that the crystals in the lymph node mainly consisted of PROJECT Q, whereas crystals in the kidney seemed to consist mainly of a metabolite of PROJECT Q.

At 100 mg/kg, food consumption decreased sporadically in females at week 1 of dosing and body weight decreased in 1 female at week 1 of dosing. Vomiting was observed in both sexes mainly at weeks 1 and 2 of dosing. Scratching and trauma were observed in 1 male. Body temperature decreased in 1 male 4 h after dosing on day 1 of dosing. In urinalysis, specific gravity decreased in both sexes, total excretion of Cl decreased in females and unclassifiable crystals in urinary sediment were observed in both sexes. In hematology, reticulocyte ratio increased in 1 male on day 15 of dosing and APTT prolonged in 1 male on day 28 of dosing. In blood chemistry, ALP and total cholesterol increased in both sexes on days 15 and/or 28 of dosing. In histopathology, atrophy and degeneration of the seminiferous tubule in the testis with absence of sperm or cell debris in the epididymis were observed in males.

At 20 mg/kg, vomiting was observed sporadically in 1 female during the dosing period; however, this was not considered toxicologically significant since it was observed in only

1 female. No test article-related changes were observed in ophthalmology at any dose level.

The changes observed at 600 or 600/300 mg/kg during the dosing period recovered or partially recovered during the 4-week recovery period. However, the following changes were observed at the end of the recovery period: decreased specific gravity in urinalysis and pathological changes observed as gross abnormalities in the kidney (calculus in the bilateral kidneys, red focus and dilatation of the renal pelvis in the left kidney and dilatation and calculus in the left ureter) in 1 female, high kidney weight and histopathological changes such as changes in the testis and epididymis in males (atrophy and degeneration of the seminiferous tubule in the testis with absence of sperm in the epididymis), changes in the kidney in 1 female (focal dilatation of the renal tubule with mononuclear cell infiltration and neutrophil infiltration, focal fibrosis, hypertrophy and desquamation in the transitional epithelium and regeneration of renal tubule), and crystal deposition in various tissues in both sexes.

In toxicokinetics, on day 1 of dosing, mean Cmax and AUC24 increased with dose level in both sexes. Following repeated dosing, in both sexes at 20 and 100 mg/kg and in females at

600 mg/kg, mean Cmax and AUC24 on day 14 of dosing were 2 to 3 times greater than those on day 1of dosing, and on day 28 of dosing were similar to those on day 14 of dosing. In males at the high dose level, mean plasma PROJECT Q concentration at 4 h after dosing on day 8 of dosing (final dosing at 600 mg/kg) was approximately 3 times greater than that on day 1 of dosing (first dosing at 600 mg/kg). Mean plasma PROJECT Q concentration before dosing on day 14 of dosing (approximately 24 h after dosing at 300 mg/kg) was approximately half that on day 9 of dosing (approximately 24 h after the final dosing at 600 mg/kg). Mean Cmax and AUC24 on day 14 of dosing were similar to those on day 28 of dosing. Mean tmax were

1 to 7 h at 20 to 600 mg/kg and tended toward prolongation by dose escalation. There was no appreciable sex difference in any parameter. The NOAEL was 20 mg/kg/day for both sexes of dogs [End-of-Text Table 3.7.2].

### Genotoxicity

The potential genotoxicity of PROJECT Q was evaluated using in vitro studies. Major findings from these studies where:

* Study Project Q-TX-0008 – PROJECT Q does not induce gene mutation in bacteria [End-of-Text Table 3.8]
* Study Project Q-TX-0009 – PROJECT Q does not induce chromosomal aberrations in CHL/IU cells [End-of-Text Table 3.8.1].
* It was concluded PROJECT Q does not exhibit genotoxicity potential in in vitro situations.

### In Vitro Reverse Mutation (Project Q-TX-0008)

To assess the potential of PROJECT Q to induce gene mutation, an in vitro bacterial reverse mutation test was performed (Project Q-TX-0008), with 5 strains of bacteria (*Salmonella typhimurium* [TA98, TA100, TA1535 and TA1537] and *Escherichia coli* [WP2*uvrA*]) with and without metabolic activation. In comparison 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, with or without metabolic activation. It was concluded that PROJECT Q does not induced gene mutation in bacteria [End-of-Text Table 3.8].

### In Vitro Chromosome Aberration (Project Q-TX-0009)

In order to assess the potential of PROJECT Q to induce chromosomal aberrations, a chromosomal aberration test was performed (Project Q-TX-0009) with cultured mammalian Chinese hamster lung cells (CHL/IU) in short-term treatments for 6 h with or without metabolic activation and continuous treatment for 24 h without metabolic activation. No significant increase in the number of cells with structural or numerical chromosomal aberrations was noted in any treatment group when compared to the negative control group. It was concluded that PROJECT Q does not induce chromosomal aberrations in CHL/IU cells,

regardless of the presence or absence of metabolic activation or treatment length [End-of-Text Table 3.8.1].

### Carcinogenicity

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

### Reproductive and Developmental Toxicity

Exploratory dose range-finding and definitive studies for embryo-fetal developmental toxicity were conducted in rats and rabbits [End-of-Text Table 3.11 and 3.12, respectively].

### Effects on Embryo-Fetal Development

### Nonpivotal Study in Rats (Project Q-TX-0010)

An exploratory study was performed to investigate the potential adverse effects of PROJECT Q on pregnant animals and embryo-fetal development (embryo-fetal death, growth retardation and malformations) in rats and to set the dose levels for the subsequent main study of the effects on embryo-fetal development in rats (Project Q-TX-0010). PROJECT Q was administered orally to 5 or 6 pregnant rats at dose levels of 0 (vehicle), 30, 300 and 2000 mg/kg/day on days 7 to 17 of gestation, the days that correspond to the period from implantation to closure of the hard palate of rat fetuses.

No death occurred in any dam. At 2000 mg/kg, scant feces were observed in 1 dam on day 8 of gestation. Decreased food consumption and suppressed body weight gain were noted in dams in the 300 and 2000 mg/kg groups, but the changes in the 300 mg/kg group were slight. No statistically significant differences were noted in body weights between the control and 300 mg/kg groups. No appreciable changes in body weights or food consumption were noted in the 30 mg/kg group. No gross pathological lesions were evident in any dam. No treatment-related effects were noted on the number of corpora lutea or implantations or preimplantation loss.

No treatment-related effects were noted on the post-implantation loss or the number, sex ratio, body weights or placental weights of live fetuses. No external malformations were observed in any fetus. It was determined that 2000 mg/kg/day would be appropriate for the high dose level in the subsequent main study of the effects on embryo-fetal development in rats [End-of-Text Table 3.11.1].

### Pivotal Study in Rats (Project Q-TX-0011)

A definitive study of embryo-fetal development was performed in rats (Project Q-TX-0011) to investigate the effects of PROJECT Q on pregnant rats and embryo-fetal development and to assess systemic exposure to PROJECT Q. PROJECT Q was administered orally to 20 pregnant rats/group at dose levels of 0 (vehicle), 30, 300 and 2000 mg/kg/day on days 7 to 17 of gestation.

No death or clinical signs were observed in any dam. Decreased food consumption and suppressed body weight gain were noted in dams in the 300 and 2000 mg/kg groups; however, the effects in the 300 mg/kg group were slight and transient (decrease in food

consumption was about 8 to 18% and 8 to 32% decrease from control animals in the 300 and 2000 mg/kg groups, respectively). No gross pathological lesions were evident in any dam. No changes suggestive of maternal toxicity were noted in the 30 mg/kg group. Pregnancy was maintained up to cesarean section in all dams. No treatment-related effects were noted on the number of corpora lutea or implantations or the pre-implantation loss (%).

No treatment-related effects were noted on the post-implantation loss (%) or the number, sex ratio, body or placental weights or the incidence of external, visceral or skeletal malformations or skeletal variations of live fetuses. The numbers of ossified bones in fetuses were comparable between the control and 2000 mg/kg groups.

Toxicokinetic data showed that PROJECT Q was not detected in plasma in the control group. Cmax and AUC24 values of PROJECT Q increased less than dose-dependently up to 2000 mg/kg after the first and last administrations; these values were almost comparable between the first and last administrations. Based on these results, the NOAELs of PROJECT Q were concluded to be 30 mg/kg/day for general toxicity in dams and 2000 mg/kg/day for the reproductive function of dams and embryo-fetal development [End-of-Text Table 3.12.1].

### Nonpivotal Study in Rabbits (Project Q-TX-0012)

An exploratory study was performed to investigate the effects of PROJECT Q on non-pregnant animals, dams and embryo-fetal development (Project Q-TX-0012).

*Study in Non-Pregnant Animals*

PROJECT Q was administered orally to 3 non-pregnant female rabbits/group, at dose levels of 100, 300 and 1000 mg/kg/day for 5 days to select dose levels for the subsequent phase of this study in pregnant animals.

No deaths occurred in any animal. Scant or no feces and a tendency to decrease in body weights and food consumption were noted in the 300 and 1000 mg/kg groups. No changes indicative of toxic effects were evident in the 100 mg/kg group. No gross pathological lesions were evident in any animal. Therefore, the dose level of 300 mg/kg/day was considered to be appropriate for the high dose of the study in pregnant animals.

On days 1 and 5 of dosing, the mean Cmax and mean AUC24 values increased less than dose proportionally up to 1000 mg/kg, except for the mean AUC24 at 1000 mg/kg on day 5 of dosing. The mean tmax values at 100, 300 and 1000 mg/kg were 1, 1 and 2 h, respectively, on day 1 of dosing. On day 5 of dosing, the mean tmax values at the corresponding doses were 1, 2 and 3 h, respectively, and were prolonged as the dose increased. The systemic exposure was almost constant during the dosing period [End-of-Text Table 3.11.2].

*Study in Pregnant Animals*

PROJECT Q was administered orally to 6 pregnant female rabbits/group at dose levels of 0 (vehicle), 30, 100 and 300 mg/kg/day on days 6 to 18 of gestation (period from implantation to closure of the hard palate of rabbit fetuses) to select dose levels for the subsequent main study.

No death occurred in any dam. Scant or no feces and no urine, probably treatment-related, were observed and decreased food consumption (about 45 to 52% decreases from control animal) was noted in the 300 mg/kg group. No clear treatment-related effects were noted in body weights of dams. No gross pathological lesions were evident in any dam. No treatment-related effects were noted on the number of corpora lutea, implantations or

pre-implantation loss (%).

No treatment-related effects were noted on the post-implantation loss (%) or the number, sex ratio, body weights or placental weights of live fetuses. No treatment-related external or visceral malformations were evident in any fetuses.

The toxicokinetic data of PROJECT Q-treated pregnant animals showed that the mean Cmax and mean AUC24 values increased more than dose proportionally on day 6 of gestation and almost dose proportionally on day 18 of gestation, up to 300 mg/kg. The mean tmax values were constant at approximately 1 h, independent of dose, on day 6 of gestation and were 1, 1, and 2 h at 30, 100 and 300 mg/kg, respectively, on day 18 of gestation. The systemic exposure was decreased as escalation of the dosing frequency and dose levels. It was determined that 300 mg/kg/day would be appropriate for the high dose level in the subsequent main study of the effects on embryo-fetal development in rabbits [End-of-Text Table 3.11.2].

### Pivotal Study in Rabbits (Project Q-TX-0013)

A definitive study of embryo-fetal development was performed in rabbits (Project Q-TX-0013) to investigate the effects of PROJECT Q on pregnant females and embryo-fetal development and to assess systemic exposure to PROJECT Q. PROJECT Q was administered orally to 17 to

20 pregnant female rabbits/group at dose levels of 0 (vehicle), 30, 100 and 300 mg/kg/day on

days 6 to 18 of gestation.

In the 300 mg/kg group, decreased food consumption (about 25 to 40% decreases from control animal), scant feces and suppressed body weight gain were noted in dams. No changes suggestive of treatment-related toxicological effects were evident on the physical condition, body weights or food consumption of dams in the 30 and 100 mg/kg group. No gross pathological lesions were observed in any dam. Total litter loss was noted in 1 dam in the 30 mg/kg group at cesarean section; however, the other dams maintained pregnancy until cesarean section and no treatment-related effects were noted on the number of corpora lutea or implantations or the pre-implantation loss (%).

No treatment-related effects were note on the post-implantation loss (%), the number, sex ratio, body or placental weights or the incidence of external, visceral or skeletal malformations or skeletal variations of live fetuses. The numbers of ossified bones in fetuses were comparable between the control and 300 mg/kg groups.

The toxicokinetic data of PROJECT Q-treated pregnant animals showed that the Cmax and AUC24 values of PROJECT Q increased more than dose-proportionally up to 300 mg/kg after the first and last administrations and both parameters decreased with repeated dosing. Based on these results, the NOAELs of PROJECT Q were concluded to be 100 mg/kg/day for general

toxicity in dams and 300 mg/kg/day for the reproductive function of dams and embryo-fetal development [End-of-Text Table 3.12.2].

### Other Toxicity Studies

Two investigative studies on immunotoxicity were conducted in rats in order to evaluate the antibody response, because immunosuppressive effect was a concern by the rat toxicity studies in which narrowing of the marginal zone of the spleen had been observed. A recovery period of 4 weeks was set to evaluate the reversibility of any observed changes and systemic exposure of PROJECT Q was analyzed. A tabulated overview of these studies is provided in End-of-Text Table 3.15.

### T-cell Independent Antibody Response in Rats (Project Q-TX-0014)

Study Project Q-TX-0014 was conducted to assess the effects of PROJECT Q on the T-cell independent antibody response (specific antibody production to DNP-ficoll). PROJECT Q was orally administered to rats for 4 weeks at doses of 0 (vehicle), 10, 30 and 300 mg/kg/day and a recovery group (recovery period of 4 weeks, at doses of 0 and 300 mg/kg/day in the dosing period) was set to evaluate the reversibility of any changes observed during the dosing period.

No test article-related changes were observed in clinical signs, body weight, food consumption, organ weight (spleen, thymus and adrenals) or necropsy finding in any test article dosing group during the dosing or recovery period. In addition, no significant difference was observed in the antibody productions of anti-DNP immunoglobulin (Ig) M or IgG antibodies in any test article administration group during the dosing period. The spleen weight, body weight gain (days 22 – 29) and production of anti-DNP IgM and IgG were significantly reduced and small spleen was observed in both male and female animals treated with the positive control cyclophosphamide monohydrate (CP) 3 mg/kg/day. In the toxicokinetic analysis, the systemic exposures of PROJECT Q were appropriately confirmed.

This data concludes that PROJECT Q does not affect the T-cell independent antibody response in this study up to 300 mg/kg/day [End-of-Text Table 3.15.1].

### T-cell Dependent Antibody Response in Rats (Project Q-TX-0015)

Study Project Q-TX-0015 was conducted to assess the effects of PROJECT Q on the T-cell dependent antibody response (specific antibody production to Keyhold Limpet Hemocyanin [KLH]). PROJECT Q was orally administered to rats for 4 weeks at doses of 0 (vehicle), 10, 30 and 300 mg/kg/day and a recovery group (recovery period of 4 weeks at doses of 0 and

300 mg/kg/day in the dosing period, 10 rats of each sex per group) was set to evaluate the reversibility of any observed changes.

No test article-related changes were observed in the antibody productions of anti-KLH IgM on day 20 and 29, or anti-KLH IgG on day 29 up to 300 mg/kg/day during the dosing period. Therefore, measurements of antibodies of the recovery group was not conducted.

No test article-related changes were noted in clinical signs, body weight, food consumption, or organ weights in any group during the dosing or recovery period. Decreases in body

weight gain, food consumption and organ weights (spleen and thymus) were observed in animals treated with positive control CP 3 mg/kg/day. Animals treated with the positive control also showed anti-KLH IgM and anti-KLH IgG levels on days 20 and/or 29 that were lower than those in the control group. In the toxicokinetic analysis, the systemic exposures of PROJECT Q were appropriately confirmed. The NOAEL of PROJECT Q in the T-cell dependent antibody response was considered to be 300 mg/kg/day for males and female rats [End-of-Text Table 3.15.2].

### 4.3.7 Local Tolerance

No local tolerance studies have been conducted with PROJECT Q.

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

PROJECT Q is a selective and potent full agonist of human and rat CB2 receptors under development for the treatment of OA pain and CLBP. CB2 receptors are not limited to the immune system, but are also found in the nervous system including microglia, spinal cord, DRG and peripheral terminal of primary afferent fibres. Though the mechanism of analgesic action of CB2 receptor agonists is not fully known, it is considered that a CB2 receptor agonist reduces afferent signals from peripheral nerves and thereby has an analgesic effect on various stimulations.

PROJECT Q induces a significant analgesic effect in preclinical models of OA pain: the AIA model and MIA model. Whereas the MIA model assesses non-inflammatory

(NSAID-resistant) pain, the AIA model is a reflection of inflammatory pain. The effective dose range in these two models was 0.1 to 1 mg/kg. The analgesic effect of PROJECT Q at

0.15 mg/kg (ED50) was comparable to diclofenac in the AIA model. No additional efficacy is obtained by dosing beyond 0.3 mg/kg in the OA pain models. Therefore, the pharmacological active dose range is considered to be 0.15 to 0.3 mg/kg. The predicted clinical effective exposures associated with a pharmacological active dose (PAD) of

* 1. mg/kg are 6.53 ng/mL for Cmax, total and 77.77 ng•h/mL for AUCinf, total. The analgesic effects of PROJECT Q in animal models are not affected by confounding factors such as decreased locomotion or other adverse effects.

PROJECT Q displayed a high degree of CB2 receptor specificity, in vitro. Data from binding assays demonstrated that PROJECT Q has a high affinity for CB2 over CB1 receptors (Ki value for human receptors of 12 nmol/L versus 3500 nmol/L for CB2 and CB1 receptors, respectively). PROJECT Q was shown to be a potent CB2 receptor agonist in vitro and a weak agonist at CB1 receptors as determined using a cAMP assay. In addition, PROJECT Q did not significantly interact with 65 of 66 different kinds of receptors, ion channels, transporters and enzymes.

Selectivity of PROJECT Q for the CB2 receptors over the CB1 receptor is supported by safety pharmacology and toxicity data. The first suggestion of an off-target CB1 receptor mediated effect, was seen in dogs at 60 mg/kg (in the safety pharmacology study of the effects on

cardiovascular and respiratory system), 531-fold above the expected clinical effective Cmax, unbound.

Since there is no mechanistic or biochemical functional biomarker for PROJECT Q, it is difficult to establish a relationship between plasma exposure and biological activity.

In dogs, a more than dose proportional increase in exposure (0.1 to 1 mg/kg) was observed, while in female rats, the exposure increased dose proportionally from 0.1 to 0.3 mg/kg and less than dose proportionally from 0.3 mg/kg to 1 mg/kg. The bioavailability ranged from 9% to 21% in dogs. In male rats, the bioavailability was 25%. PROJECT Q has a relatively low brain-to-plasma ratio. Considering the relative low bioavailability in preclinical species, low bioavailability in humans may have serious implications for drug-drug interactions.

PROJECT Q is mainly metabolized by CYP3A4/5, and as a consequence, is very vulnerable to clinical drug-drug interactions. In addition, it is a substrate for P-gp, which may also increase its potential for drug-drug interactions, as well as reduce bioavailability, and may explain low CNS penetration.

The appropriate rodent (rat) and non rodent (dog) species were selected for the safety pharmacology and toxicology studies based on their metabolic profiles compared to human cryopreserved hepatocytes.

Toxic findings for PROJECT Q were only seen at high exposures and included side effects such as:

* + 1. CB1 cross-reactivity/CNS signs:
       1. Reduced body temperature (rats and dogs)
       2. CNS and other clinical signs (dogs)
    2. Crystal deposition in various organs (dogs)
    3. Renal toxicity (rats and dogs): renal changes in dogs may be associated with crystal formation, but those in rats are not.
    4. Liver toxicity (rats and dogs): the association with crystals is not clear.
    5. Testicular changes (dogs)
    6. Effects on cardiovascular and respiratory system (dogs): risk in humans is low.
    7. Effects on the spleen (rats): of little toxicological significance.
    8. No teratogenic and genotoxic potential.

*CB1 Receptor Cross-reactivity/CNS Signs*

Transient decreases in body temperature were observed in rats given 2000 mg/kg and dogs treated with a single dose of 60 mg/kg or more. The decreases in body temperature may be related to an off-target CB1 receptor effect, as CB1 receptor agonists are known to cause a rapid and significant decreases in body temperature [Rawls et al, 2006]. No clinical signs

were apparent in rats. In dogs, vomiting and scratching were observed at ≥ 60 mg/kg. Evidence of neuronal inhibition (decrease in spontaneous activity, incomplete eyelid opening and drowsiness/somnolence) and activation (staggering, twitch, tremor and spasticity) were noted at the higher doses (≥ 600 mg/kg). Some of these CNS-related signs may be associated with CB1 receptor cross-reactivity [Huestis, 2002].

*Crystal Deposition in Various Organs*

In the 4-week dog study, crystals were noted in urinary sediments at ≥100 mg/kg, and histopathological examination revealed that crystals were deposited in various tissues at 600/300 mg/kg and/or 600 mg/kg. Crystals were found in the renal papilla/ ureter and tubule in the kidney, as well as in Kupffer cells in liver, and giant cells in lower digestive tracts, urinary bladder, lung, prostate, skin, and lymphoid tissues. The giant cells of the organs and the Kupffer cells in the liver are the macrophages of the specific organs and serve to digest foreign bodies. After a 4-week recovery period, crystals were still observed in some tissues, although they tended to recover in terms of severity and frequency, but were not noted in the urinary sediment. Tissue crystals were not found in dogs after single administration up to 1500 mg/kg, or after 1-week administration in exploratory 1-week study up to 600 mg/kg. In addition to the dependency to dosing period, crystal deposition were considered to be related to the systemic exposure of PROJECT Q. That is to say, the AUC24 values at day 28 of dosing associated with tissue crystal formation ranged from 482,898 to 1,859,686 ng·h/mL for individual dogs (roughly correlating with severity); 351,661 ng·h/mL for the mean of

100 mg/kg group animals with urinary crystals without tissue crystals; and 23,126 ng·h/mL for the mean of 20 mg/kg group without urinary and tissue crystals.

*Renal Toxicity*

Slight renal toxicity occurred in rats after 4-week repeat dosing at 2000 mg/kg, which was associated with nephrotoxicity parameters (BUN). In addition, more pronounced renal toxicity such as granuloma or fibrosis occurred in dogs at 600/300 and/or 600 mg/kg, which could possibly be related to crystal deposition. The histopathological changes in the kidney did not fully recover during the 4-week post treatment period.

*Liver Toxicity*

Repeated dosing in rats was associated with a minor decrease in albumin at ≥300 mg/kg, and slight prolongation of PT and APTT at 2000 mg/kg in male. Since the changes were minor, and there was no evidence of bleeding, these findings were considered of limited clinical relevance. In the dog repeated dose study, increased ALP and cholesterol were noted at ≥100 mg/kg; and marked increases in AST, ALT, and bilirubin in some animals at 600/300 and/or 600 mg/kg from week 2 onwards. Very slight crystal deposition in Kupffer cell and hyperplasia of bile duct were observed at histopathology. Blood chemistry values returned to normal values after the 4-week recovery period, whereas the crystal deposition in Kupffer cell was still present. There was no evidence of clear hepatic cell death that may be related to the marked increases in liver enzymes.

*Testicular Change*

In the dog 4-week repeated dose study, degeneration and atrophy of seminiferous tubules in the testes, accompanied by absence of sperm and the presence of cell debris in the epididymis, were observed at ≥100 mg/kg. The changes were still observed at 600/300 mg/kg after 4-week recovery, although they tended to recover in terms of severity. The recovery period of 4-weeks may not have been long enough, because a full spermatogenic cycle in dogs takes about 62 days. No testicular findings were seen in the 4-week rat study. The testicular changes possibly reflect PROJECT Q-induced hormonal changes, since similar testicular findings are reported in immature dogs [Goedken et al, 2008]. Hormonal changes could be related to off target activity of PROJECT Q on the CB1 receptor. In this respect, the cannabinoid delta-9-tetrahydrocannabinol is reported to induce male infertility by lowering luteinizing hormone (LH) levels in hypothalamus, and thereby reducing testosterone production by the Leydig cells [Fasano et al, 2009]. Additionally, stress has been reported to interfere with sex hormones, which may have added to the severity of the findings, particularly at 600/300 mg/kg. However, direct testicular toxicity cannot be excluded.

*Effects on Cardiovascular and Respiratory Systems*

An inhibitory effect of PROJECT Q on the hERG current (22%) was revealed at 30 mcM, whereas no changes were noted at a 10-fold lower concentration (1,537 ng/mL, exposure ratio of 759-fold based on expected clinically effective Cmax,u). In an in vivo study, QTc prolongation was seen in the cardiovascular and respiratory system of dogs at ≥ 600 mg/kg. QTc prolongation at these high doses might possibly be associated with the inhibitory effect of PROJECT Q on an in vitro hERG current, or may be secondary to severe hypothermia [van der Linde et al, 2008] (maximum decrease -1.9°C). In addition arterial blood PaCO2 was increased and arterial blood pH decreased, without changes in respiration rate, PaO2 or SaO2. Slight changes in electrolytes were observed at ≥ 60 mg/kg. ECG changes (QTc and QRS prolongation) observed in female dogs treated at 600 mg/kg for 4-weeks might be related to aggravated general conditions. The ratio between the exposure at which effects occurred in dogs and the anticipated clinical effective exposure is very large, based on expected clinically effective Cmax,u (2674-fold for the effect). The safety ratio at 60 mg/kg (NOAEL for QTc and QRS prolongation) is 531-fold to the expected clinically effective Cmax,u. Therefore, the risk of cardiovascular or respiratory changes at therapeutic exposures in humans is considered low.

*Effects on the Spleen*

In the 1-week and 4-week rat repeated dose studies, very slight to moderate narrowing of the marginal zone of the spleen was observed at ≥100 mg/kg in male rats and ≥300 mg/kg in female rats, but not after the 4-week recovery period. In two 4-week functional immunotoxicity studies in rats (T-cell dependent and independent antibody response), no significant change was noted in antibody response up to 300 mg/kg. Cannabinoids have been reported to possess immunomodulatory effects [Klein and Cabral, 2006] and CB2 receptors are expressed in rat spleen in both the macrophages and B cells [Tanasescu and

Constantinescu, 2010; Cesta, 2006]. The effects of PROJECT Q on the spleen may be related to the pharmacodynamic effects as a CB2 receptor agonist. However, as there was no significant effect noted in antibody reaction for T-cell independent and T-cell dependent antigens, this finding was considered of little toxicological significance.

In conclusion, PROJECT Q showed robust signs of efficacy in models of both inflammatory (AIA) and non-inflammatory (MIA) models of pain, indicating a potential use in the treatment of OA pain and CLBP. There are no safety or toxicological findings that were judged to prohibit the initiation of an PROJECT Q SAD study.

#### List of References

Cesta MF. Normal structure, function, and histology of the spleen. Toxicol Pathol. 2006;34:455-65.

Fasano S, Meccariello R, Cobellis G, Chianese R, Cacciola G, Chioccarelli T, et al. The endocannabinoid system: an ancient signaling involved in the control of male fertility. Ann NY Acad Sci. 2009;1163:112-24.

Goedken MJ, Kerlin RL, Morton D. Spontaneous and age-related testicular findings in beagle dogs.

Toxicol Pathol. 2008;36:465-71.

Huestis MA. Cannabis (Marijuana)-Effects on human behavior and performance. Forensic Sci Rev.

2002;14:15-60.

Klein TW, Cabral GA. Cannabinoid-induced immune suppression and modulation of antigen presenting cells. J Neuroimmune Pharmacol. 2006;1:50-64.

Rawls SM, Tallarida RJ, Zisk J. Agmatine and a cannabinoid agonist, WIN 55212-2, interact to produce a hypothermic synergy. Eur J Pharmacol. 2006;553:89-98.

Tanasescu R, Constantinescu CS. Cannabinoids and the immune system: an overview.

Immunobiology 2010;215:588-97.

van der Linde HJ, Van Deuren B, Teisman A, Towart R, Gallacher DJ. The effect of changes in core body temperature on the QT interval in beagle dogs: a previously ignored phenomenon, with a method for correction. Br J Pharmacol. 2008;154:1474-81.