**A 4-Week Repeated Oral Dose Toxicity Study of PROJECT C in Dogs Followed by a 4-Week Recovery Period**

**11 SUMMARY**

PROJECT C was suspended in 0.5 w/v% methylcellulose (MC) solution and orally administered once daily for 4 weeks at dose levels of 0 (vehicle control), 1, 3, 10, and 100 mg/kg to 4 male and 4 female beagle dogs per group in order to investigate its toxicity. Three males and three females were added to the highest dose group (100 mg/kg) to assess the reversibility of toxicity during a subsequent 4-week recovery period. Systemic exposure to PROJECT C was also assessed. The following observations and examinations were performed: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, gross pathology, organ weights, histopathology, and toxicokinetics.

At 100 mg/kg, 1 male was sacrificed on Day 14 of dosing, animal showed mainly a severe decrease in spontaneous activity, vomiting, somnolence, and ataxic gait. Decreased food consumption was noted from Day 4 of dosing, and body weight on Day 14 of dosing was decreased by approximately 25% from that on Day -1. High erythrocyte count, hemoglobin concentration, hematocrit value, and high leukocyte, neutrophil, and monocyte counts were noted in hematology and high alanine transaminase, total protein, albumin, total cholesterol, glucose, and urea nitrogen, and low sodium, potassium, and chloride were noted in blood chemistry on Day 13 of dosing and/or at sacrifice. In histopathology, atrophy of the thymus and a decrease in pancreatic zymogen granules were observed. These changes were almost identical to those in the scheduled sacrificed animals. It was considered to be the deterioration of general condition in accordance with the test article administration.

In the surviving animals, no test article-related changes were noted at 1 or 3 mg/kg.

At 10 mg/kg, vomiting was observed in 1 male mainly immediately after dosing or 1 to 2 hours after dosing between Weeks 1 and 3 of dosing, and salivation was observed in males immediately after dosing.

At 100 mg/kg, vomiting was observed in males and females mainly before dosing, immediately after dosing, or 1 to 2 hours after dosing between Weeks 1 and 3 of dosing, and salivation was observed in males and females mainly immediately after dosing or 1 to 2 hours after dosing during the dosing period. Somnolence was observed in males and 1 female mainly 1 to 2 or 4 to 6 hours after dosing at Weeks 1,2, and/or 3 of dosing, and ataxic gait was observed in males and females mainly 1 to 2 or 4 to 6 hours after dosing at Week 1 of dosing. A decrease in spontaneous activity was observed in males and females mainly 1 to 2 or 4 to 6 hours after dosing at Weeks 1 and 2 of dosing. Decreased food consumption was noted in males and females, and body weights were decreased by approximately 10% to 20% from that on Day -1 of dosing. A low heart rate, and prolongation of QT and QTc in 1 male were noted. High urinary bilirubin in 1 male, high urinary glucose in 1 female, low sodium excretion in females, and low potassium and chloride excretion in males and females were noted. Low erythrocyte count, hemoglobin concentration and hematocrit value in males, high erythrocyte count, hemoglobin concentration and hematocrit value in females, high platelet count in 1 female, low lymphocyte count in females, and high fibrinogen in males were noted. High alanine transaminase, globulin, and sodium were each noted in 1 female. High total protein, albumin, glucose, and chloride in females, high total cholesterol in 1 male and females, high urea nitrogen in 1 male, and low sodium and low chloride in 1 male were noted. Small-sized thymus and low thymus weights was observed in 1 male and 1 female, and moderate atrophy of the thymus and a slight decrease in pancreatic zymogen granules in histopathology were observed in these animals. Low testes weights were noted in 1 male, and marked atrophy of the seminiferous tubules in the testes and absence of the sperm in the epididymides in histopathology were observed in the male.

No test article-related abnormalities in ophthalmology were observed at any dose.

During the recovery period, no test article-related changes were noted in any examination. The changes observed in the testes and epididymides at the end of the dosing period were not observed at the end of the recovery period, and other changes noted during the dosing period recovered by Week 4 of recovery.

In toxicokinetics, mean TK parameters (tmax, Cmax, and AUC24) increase with increasing dose level. After repeated dosing, mean TK parameters were approximately the same at 1, 3, and 10 mg/kg except for mean AUC24 values in males at 10 mg/kg, and mean AUC24 values in males at 10 mg/kg increased after repeated dosing. Mean TK parameters at 100 mg/kg increased or tended to increase after repeated dosing except for mean tmax values in females, and mean tmax values in females were approximately the same after repeated dosing. There was no clear sex difference in any TK parameter.

The tmax, Cmax, and AUC24 values for PROJECT C are shown in the table below.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dose Level (mg/kg/day) | | 1 | | 3 | | 10 | | 100 | |
| Males | Females | Males | Females | Males | Females | Males | Females |
| tmax (h) | Day 1 | 1.3 | 1.4 | 2.3 | 2.3 | 1.0 | 1.6 | 4.4 | 4.3 |
| Day 14 | 2.3 | 1.0 | 1.3 | 1.5 | 1.8 | 1.3 | 2.4 | 1.6 |
| Day 28 | 1.5 | 1.0 | 2.0 | 1.3 | 2.0 | 1.5 | 7.8 | 3.3 |
| Cmax (ng/mL) | Day 1 | 78.25 | 71.56 | 179.60 | 174.73 | 241.68 | 278.73 | 962.70 | 904.44 |
| Day 14 | 93.50 | 108.45 | 187.12 | 171.48 | 439.75 | 361.14 | 2384.15 | 1486.04 |
| Day 28 | 105.34 | 75.54 | 246.78 | 234.81 | 310.72 | 435.84 | 1702.84 | 1970.81 |
| AUC24  (ng·h/mL) | Day 1 | 630.16 | 499.03 | 1724.56 | 1534.69 | 1425.89 | 3256.14 | 7620.24 | 6541.89 |
| Day 14 | 763.36 | 723.03 | 1729.88 | 1686.12 | 3853.56 | 3734.45 | 28785.41 | 21494.69 |
| Day 28 | 845.20 | 543.47 | 2338.52 | 1846.88 | 3025.23 | 4657.33 | 21551.12 | 27895.49 |

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level of PROJECT C was 3 mg/kg/day for males and 10 mg/kg/day for females because vomiting and salivation in males at 10 and 100 mg/kg/day and in females at 100 mg/kg/day, decreases in food consumption and body weight in males and females at 100 mg/kg/day, and low lymphocyte count and high total protein, albumin, and glucose in females at 100 mg/kg/day were noted The test article-related changes noted during the dosing period recovered by Week 4 of recovery.