**A 4-Week Repeated Oral Dose Toxicity Study of Project D in Cynomolgus Monkeys with a 4-Week Recovery Period**

**11 SUMMARY**

Project D was suspended in 0.5 w/v% methylcellulose solution and orally administered once daily for 4 weeks at dose levels of 0, 1, 3, and 10 mg/kg (as PROJECT D) to 4 male and 4 female cynomolgus monkeys (3 to 7 years of age) per group in order to investigate its toxicity. Three males and three females were added to the 3 and 10 mg/kg groups to assess the reversibility of toxicity during a subsequent 4-week recovery period. Systemic exposure to PROJECT D was also assessed. The following observations and examinations were performed: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, lactic acid measurement, gross pathology, organ weights, histopathology, electron microscopy, and toxicokinetics.

At 10 mg/kg, 1 male was sacrificed due to moribundity approximately 7 hours after dosing on Day 24 of dosing. In this male, vomiting was observed mainly at approximately 1 or 6 hours after dosing on a total of 9 days during Weeks 2 and 3 of dosing, and a slight decrease in spontaneous activity was observed since Day 16 of dosing. Lateral position, hypothermia, and suppressed response to stimulation were observed approximately 6 hours after dosing on Day 24 of dosing. Body weight was decreased on Day 21 of dosing by approximately 10% from that on Day -1 of dosing. A low heart rate and prolongation of QT and QTc intervals were noted. A high lymphocyte vacuolation ratio was noted. In histopathology, the following changes were observed: vacuolation of macrophages in the sternal and femoral bone marrow, thymus, spleen, submandibular and mesenteric lymph nodes, and Peyer’s patch; vacuolation in the myocardium; accumulation of foam cells in the lung; hypertrophy of Kupffer cells in the liver; hyaline/erythrocyte casts and hypertrophy of podocytes in the glomeruli in the kidney; vacuolation of neurons in the brain. Additionally, high leukocyte count, neutrophil count, aspartate transaminase, alanine transaminase, glucose and urea nitrogen, and low chloride in blood were noted at sacrifice.

No test article-related changes were noted at 1 mg/kg.

At 3 mg/kg and above, increased counts of leukocyte, lymphocyte, monocyte, eosinophil, and large unstained cell were noted in males and/or females, in addition, basophil count increased in males. A high aspartate transaminase in males, high alanine transaminase and triglycerides in males and/or females, and low sodium and chloride in males and/or females were noted in blood. In histopathology, vacuolation of macrophages in the sternal and/or femoral bone marrows, thymus, spleen, submandibular and/or mesenteric lymph nodes, and Peyer’s patch in males and/or females, and accumulation of foam cells in the lung in males and females were observed.

At 10 mg/kg, a slight decrease in spontaneous activity was observed in males and females from Day 24, 27, or 28 of dosing to Day 29 of dosing. Body weight decreased in males and females at 10 mg/kg during the dosing period [body weights on Day 28 of dosing were decreased by approximately 8% to 24% from those on Day −1 of dosing]. Additionally, vomiting was observed in males and females, mainly at approximately 1 or 6 hours after dosing on 1 to 18 days, and slight salivation was observed in males and females on 1, 2, or 3 days. A low heart rate in males and females, prolongation of QT interval in males and females, and prolongation of QTc interval in males were noted. Occult blood reaction and high urinary glucose in males, high urinary protein in males and females, and low sodium and chloride excretion in females were noted. In blood chemistry, high fibrinogen in females, high total bilirubin in males and females, and high urea nitrogen and potassium in males were noted. High lactic acid was noted in males and females at 10 mg/kg at 6 and/or 24 hours after dosing on Days 14 and/or 28 of dosing. A high lymphocyte vacuolation ratio in blood was noted in males and females. High heart, kidney, and lung weights were noted. In histopathology, hypocellularity in the sternal bone marrow in males and females, atrophy of lymphoid follicles in the spleen in females, vacuolation in the myocardium in males and females, hypertrophy of Kupffer cells in the liver in males and females, hypertrophy of podocytes in the glomeruli in the kidney in males, degeneration of the follicles in the thyroid in males, vacuolation of macrophages in the vagina in females, and vacuolation of neurons in the brain in males and females were observed. In electron microscopy, intracytoplasmic single membrane-bound lamellar bodies were observed in the Kupffer cells and hepatocytes in males and females, and the epithelia of the renal tubules and podocytes in the glomeruli in males. Based on the characteristic ultrastructure, the systemic vacuolation detected microscopically was diagnosed as phospholipidosis.

No test article-related abnormalities in ophthalmology or gross pathology were noted in the 3 or 10 mg/kg group.

In the recovery period, decreased food consumption was noted in females at 10 mg/kg on Days 1, 2, 6, and 8 of recovery, but no abnormalities were noted thereafter. In histopathology, very slight accumulation of foam cells in the lung was observed in males at 10 mg/kg; however, the severity and incidence of this change became milder, and no test article-related changes in other organs or tissues were observed in histopathology. Therefore, test article-related changes noted during the dosing period or at the end of the dosing period recovered or tended to recover.

Toxicokinetics parameters of PROJECT D are shown in the table below.

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| --- | --- | --- | --- | --- | --- |
| Day | Dose level (mg/kg/day) | Sex | Cmax (ng/mL) | tmax (h) | AUC24 (ng·h/mL) |
| Day 1 | 1 | Male | 345 | 6.0 | 3020 |
| Female | 352 | 4.5 | 3570 |
| 3 | Male | 1080 | 6.0 | 10000 |
| Female | 1550 | 5.4 | 13600 |
| 10 | Male | 3100 | 5.7 | 31000 |
| Female | 3670 | 5.4 | 40600 |
| Day 14 | 1 | Male | 1330 | 5.5 | 14000 |
| Female | 784 | 6.0 | 9140 |
| 3 | Male | 3530 | 6.0 | 34500 |
| Female | 3670 | 5.7 | 34500 |
| 10 | Male | 8690 | 5.1 | 99200 |
| Female | 10200 | 5.4 | 146000 |
| Day 28 | 1 | Male | 1310 | 6.0 | 12900 |
| Female | 924 | 6.0 | 9400 |
| 3 | Male | 2960 | 6.0 | 31800 |
| Female | 2780 | 6.0 | 29300 |
| 10 | Male | 7940 | 6.0 | 107000 |
| Female | 10500 | 5.7 | 132000 |

tmax values were from 4.5 to 6.0 hours in all dose groups. Cmax and AUC24 values increased almost dose proportionally for both sexes. With repeated dosing, Cmax and AUC24 values on Days 14 and 28 of dosing were higher than those on Day 1 of dosing at each dose level for both sexes. There was no difference between Day 14 and Day 28 of dosing regarding Cmax and AUC24. No clear differences were observed in Cmax or AUC24 values in either sex at any dose.

It was concluded that, under the conditions of this study, the NOAEL was 1 mg/kg/day as PROJECT D for males and females.