**A 4-Week Repeated Oral Dose Toxicity Study of Project E in Cynomolgus Monkeys Followed by a 4-Week Reversibility Study**

**11 SUMMARY**

Project E was suspended in vehicle, water for injection, and was administered orally (gavage) once daily at dose levels of 0 (vehicle), 6, 20, 60, and 150 mg/kg/day as PROJECT E to male and female cynomolgus monkeys for 4 weeks under non- fasted conditions in order to investigate its toxicity. The animals in the control group received water for injection. The animals were 3 to 7 years old at the start of dosing. Four males and four females were assigned to each group for toxicity evaluation during a 4-week treatment period. Three additional males and females per group were assigned to the 60 and 150 mg/kg/day groups for reversibility evaluation during a subsequent 4-week recovery period. Systemic exposure to PROJECT E was also evaluated. The following observations and examinations were performed: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, CK and LDH isoenzyme activities (total and each isoenzyme) and cardiac troponin I and T, gross pathology, organ weights, and histopathology.

No test article-related changes were noted at 6 mg/kg.

At 20 mg/kg, soft stool and/or diarrhea were observed in 2 males. Increased total CK and CK-MM activities were noted in 1 male at Week 2.

At 60 mg/kg, soft stool and/or diarrhea were observed in all males and 6 females. Vomiting was observed in 4 males and 4 females most frequently during Week 1. Salivation was observed in 6 males and 5 females for 1 to 18 days from Day 7. Transiently decreased food consumption was noted in 2 females at Week 1. Increased liver weight was noted in 1 male and 1 female. Hypocellularity was observed in the sternal bone marrow in 1 male.

At 150 mg/kg, soft stool and/or diarrhea were observed in all males and all females. Vomiting was observed in all males and all females most frequently during Week 1. Salivation was observed in 6 males and all females for 1 to 16 days from Day 9. Transiently decreased food consumption was noted in 2 females at Week 1. Decreased albumin and total cholesterol and increased triglycerides were noted in 1 male at Weeks 2 and/or 4. Increased CK-MB activity in 2 males, increased total CK and CK-MM activities in one of these males, and increased levels of troponin T and troponin I in another male were noted at Week 2. Increased liver weight was noted in 2 males and 3 females.

During the recovery period, no test article-related changes were noted at 60 or 150 mg/kg in any examination, except for the hypocellularity observed in the sternal and femoral bone marrow in 1 male at 150 mg/kg. Reversibility could not be evaluated for the decreased albumin and total cholesterol and increased triglycerides because the animal that showed the finding was necropsied at the end of the dosing period.

In toxicokinetics, the results of incurred sample reanalysis (ISR) on Days 1 and 14 did not meet the acceptance criteria. Therefore, the toxicokinetics data were regarded as reference and are summarized below.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dose Level (mg/kg/day) | | 6 | | 20 | | 60 | | 150 | |
| Males | Females | Males | Females | Males | Females | Males | Females |
| tmax (h) | Day 1 | 2.5 | 2.0 | 4.3 | 4.5 | 4.6 | 4.6 | 3.9 | 3.9 |
| Day 14 | 2.5 | 2.0 | 3.3 | 4.0 | 4.9 | 2.9 | 3.9 | 3.4 |
| Day 28 | 3.5 | 2.0 | 2.8 | 3.0 | 2.9 | 3.1 | 3.1 | 3.7 |
| Cmax (ng/mL) | Day 1 | 988.0 | 739.9 | 4678.0 | 2766.3 | 7479.1 | 8350.8 | 7766.4 | 9031.4 |
| Day 14 | 1347.4 | 386.9 | 2445.1 | 2490.0 | 7210.0 | 6414.5 | 15228.2 | 10915.0 |
| Day 28 | 931.8 | 657.9 | 1807.5 | 2641.2 | 6946.8 | 6114.7 | 14553.0 | 10631.6 |
| AUC24  (ng·h/mL) | Day 1 | 4783.0 | 3966.6 | 29876.5 | 18891.9 | 71172.0 | 71020.7 | 77806.1 | 81425.6 |
| Day 14 | 6425.8 | 2572.6 | 16017.0 | 13432.6 | 73637.5 | 46710.1 | 151508.8 | 104594.7 |
| Day 28 | 4557.5 | 2879.6 | 11799.8 | 13664.3 | 56794.4 | 43355.0 | 128936.6 | 100662.9 |

In a 13-week study of Project E in cynomolgus monkeys (Study No.: SBL500-088, dose levels: 0, 6, 20 and 150 mg/kg/day) [[Yamamoto, 2014]](#_bookmark67), toxicokinetics analysis was conducted using a modified method, and ISR was performed and the acceptance criteria were met. The mean Cmax and AUC24 of PROJECT E increased as the dose level increase from 6 to 20 mg/kg and from 20 to 150 mg/kg on Days 1, 14, and 28. Mean Cmax and AUC24 were not influenced by repeat dosing except at 20 mg/kg. At 20 mg/kg/day, both parameters tended to decrease by repeat dosing. Mean tmax was delayed by dose increasing, but not influenced by repeat dosing. TK parameters did not show a sex difference. Therefore, it was considered that systemic exposure to PROJECT E in the present study would also have increased as the dose level increased from 6 to 20 mg/kg and from 20 to 150 mg/kg in the present study, that these parameters might have not been influenced by repeated dosing, and that there would have been no clear difference between sexes in any parameter.

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was 6 mg/kg/day for males and 20 mg/kg/day for females because soft stool and/or diarrhea were observed in males at 20 mg/kg and in females at 60 mg/kg. Females at 60 mg/kg also showed vomiting, decreased food consumption, and increased liver weight. After the 4-week recovery period, no abnormalities were noted except for the hypocellularity in the bone marrow.