**A 4-Week Oral Dose Toxicity Study of Project Y in Dogs with an 8-Week Untreated Recovery Period**

1. **SUMMARY AND CONCLUSION**

This study was designed to evaluate the potential toxicity of PROJECT Y when administered orally to beagle dogs (3 animals/sex/group) for 4 weeks at dose levels of 3, 10 and 300 mg/kg/day and to determine the toxicokinetic profile of PROJECT Y. These doses are equivalent to 6, 20 and 600 mg/kg of Project Y, respectively (PROJECT Y:TC- 5 = 1:1). The dosing formulations were prepared by suspending Project Y in water for injection (vehicle). Control animals (3 animals/sex) received the vehicle alone in a similar manner. The reversibility of any effects was also assessed following an 8-week untreated recovery period in 3 animals/sex in the 300 mg/kg group.

Observations and measurements performed were as follows: clinical observation, body weight, food consumption, ophthalmology, electrocardiography, hematology, clinical chemistry, testosterone measurement, urinalysis, gross pathology, organ weight and histopathology. Measurements for plasma concentrations of PROJECT Y were also conducted.

All animals survived the duration of the study.

The pharmacological actions of the test article, which were not adverse effects, were observed in the genital systems as follows. In males, a dose-dependent decrease in testosterone was recorded in the 3, 10 and 300 mg/kg groups. Decreases in the absolute and relative weights of the testes, epididymides and prostate were noted in the 10 and 300 mg/kg groups. Histopathological examination revealed atrophy of the seminiferous tubules and hypertrophy or hyperplasia of interstitial cells in the testes in the 10 and 300 mg/kg groups, cell debris in the lumens of the epididymides in the 10 mg/kg, decreased sperm and atrophy of the epithelium in the epididymides in the 10 and 300 mg/kg groups, and atrophy of the epithelium in the prostate in the 3, 10 and 300 mg/kg groups. In females, decreases in the absolute and relative weights of the uterus were noted in the 3, 10 and 300 mg/kg groups. Slight atrophy of the uterus was observed histopathologically in the 300 mg/kg group.

Loose stools were observed for 1 female in the 10 mg/kg group and for 1 male in the 300 mg/kg group. Muddy stools were also observed for 1 female in the 10 mg/kg group. Vomitus was observed for 1 female in the 10 mg/kg group and for 2 males and 2 females in the 300 mg/kg group.

No treatment-related abnormalities were noted in ophthalmology or electrocardiography during the dosing or recovery period.

The hematological examination revealed decreases in the red blood cell count, hemoglobin concentration and hematocrit in 1 female in the 300 mg/kg group.

The clinical chemistry examination revealed increases in ALP, total cholesterol, triglycerides and phospholipids in both sexes in the 300 mg/kg group.

In the organ weight measurements, an increase in relative liver weight was noted in males in the 300 mg/kg group.

In individual data, 1 male in the 300 mg/kg group exhibited a distinctively higher exposure level than the other animals in the same group, especially on Day 28 of dosing. This male exhibited the following findings: clinical signs (scant/no feces), decreases in body weights, food consumption, serum sodium and chloride, urinary sodium-, potassium- and chlorideexcretion and specific gravity of urine, shortened APTT, and increases in hemoglobin concentration, MCHC, platelet count, total bilirubin, urea nitrogen and serum potassium and histopathological lesions (atrophy of the thymus, atrophy of the hepatocytes, vacuolation of the proximal tubular epithelium and dilatation of the tubules in the kidneys). These were most likely related to deterioration of general physical condition.

The Tmax values were around 2 hours post-dosing in the 3, 10 and 300 mg/kg groups and tended to be delayed in the 300 mg/kg group. The Cmax and AUC0-24h values in both sexes in the 3, 10 and 300 mg/kg groups increased with increasing dose levels. During repeated dosing, the Cmax and AUC0-24h values were almost constant or slightly decreased. There was no appreciable gender difference.

During the 8-week recovery period, the pharmacological effects on the genital systems (decreased testosterone, histopathological findings in the testes, epididymides, prostate and uterus, and their decreased weights) as well as the other treatment-related changes were recovered or tended to recover.

In conclusion, the no observed adverse effect level (NOAEL) of PROJECT Y was considered to be 3 mg/kg/day under the conditions of this study, on the basis of loose stools, muddy stools and vomitus at 10 and 300 mg/kg/day, and decreases in erythrocytic parameters and increases in ALP, serum lipid parameters and liver weight and deteriorated physical condition at 300 mg/kg/day. The toxicological and pharmacological findings induced by PROJECT Y were recovered or tended to recover.